Organized by the Fodder Crops Section of Eucarpia - the European Association for Research on Plant Breeding

Organizing Committee:

A.P.M. den Nijs (chairman) – Centre for Plant Breeding Research (CPO), Wageningen
A.J.P. van Wijk (secretary) – VanderHave Research, Vlijmen
L.J. Beerepoot – Barenbrug Holland BV, Oosterhout
A.M. van de Bunte – International Agricultural Centre (IAC), Wageningen
A. Elgersma – CPO, Wageningen
E.J.L. Hotke-Staal – IAC, Wageningen

Financial support is gratefully acknowledged from:

Barenbrug Holland BV – Netherlands
Cebeco Zaden BV – Netherlands
Green Genetics BV – Netherlands
J. Joordens' Zaadhandel BV – Netherlands
Mommersteeg International BV – Netherlands
Van Engelen Zaden BV – Netherlands
Royal Van Der Have – Netherlands
Zelder BV – Netherlands
Franken BV/Westrup A/S – Netherlands/Denmark
H.U. Hege Saatzuchtmaschinen – Germany
Wintersteiger GmbH & Co – Austria
J. Haldrup A/S – Denmark
Damas Maskinfabrik A/S – Denmark
PBS International – United Kingdom

Ministry of Agriculture, Nature Management and Fisheries
Eucarpia - the European Association for Research on Plant Breeding

Eucarpia Secretariat:
M. Mesken
c/o Wiersum
Rendierweg 10
NL 8251 PD Dronten
Netherlands

Fodder crops breeding: Achievements, novel strategies and biotechnology

Proceedings of the 16th Meeting of
the Fodder Crops Section of Eucarpia,
Wageningen, Netherlands, 18-22 November 1990

A.P.M. den Nijs and A. Elgersma (Editors)

Pudoc Wageningen 1991

CIP-data Koninklijke Bibliotheek, Den Haag

Fodder

Fodder crops breeding: achievements, novel strategies and biotechnology / A.P.M. den Nijs and A. Elgersma eds.. - Wageningen : Pudoc. - Ill.
Proceedings of the 16th meeting of the Fodder Crops Section of Eucarpia, Wageningen, The Netherlands, 18-22 November 1990. - With index
ISBN 90-220-1029-5 bound
NUGI 835
Subject heading: fodder crops.

ISBN 90-220-1029-5
NUGI 835

Printed in the Netherlands.

FOREWORD

The volume which lies before you has been arranged around the two main themes of the conference, i.e., the achievements so far, and the use of a wide array of new breeding techniques including biotechnology. The thematical treatment is preceded by an introduction, and finally attention is focussed on the perspectives of these techniques for reaching future goals of breeders of forage crops.

The themes were addresssed by invited specialists in main papers, and many participants provided detailed extra information on posters. The two-page summaries of the posters follow directly the main papers. The first theme was also the subject of rather lively discussions in four parallel workshops, of which short reports are included. We have strived to keep publication time of these proceedings as short as possible and we like to thank all involved in their help with this effort. We do hope this volume will prove to be a useful account of the state of the art in our fast-moving and challenging area of work.

A.P.M. den Nijs and A. Elgersma
Editors

CONTENTS

Posters

PREFACE

Following the invitation to the section to hold its 16th meeting in the Netherlands in 1990 some 120 participants from 20 countries assembled at the International Agricultural Centre at Wageningen from the 18th to the 22nd of November to consider the theme "Fodder Crop Breeding; Achievements, Novel Strategies and Biotechnology".

The venue for our meeting was a very appropriate setting for our deliberations, for the Netherlands, and more specifically the various research centres located in Wageningen, have a long history of involvement with fodder crop breeding and research. This has evolved in conjunction with the Agricultural University which has played such an important role in education and research in the Netherlands. Indeed its role has spread very far afield and the International Agricultural Centre, which provided us with such excellent facilities for our conference, is testimony to the most creditable reputation that it has achieved.

Plant breeding is now at a cross roads in many countries. In several member states of the European Community for example there have been major changes in the way that plant breeding is organized and funded. This has arisen because of increasing commercial pressures and the need to exploit the rapid technological advances that are now being made. In so doing we have to consider the problems of surpluses in some countries and deficits of agricultural production in others. In addition there is the need for us to give greater consideration to the environmental implications of our breeding activities. Against this scene it is thus very appropriate that we met to assess what fodder crop breeders have achieved and where the new technologies may assist us in improving the efficiency of our breeding methods and the products which we produce.

The programme of the meeting was arranged into four main sessions. The first reviewed the progress that had been made in fodder crop breeding in the various geographic regions of Europe. Inevitably the complexity of the crop dictated as to how one assessed progress; whether it be in terms of total annual yield, persistency, winter-hardiness or quality for example, it was most pleasing to note that progress had indeed been achieved. In relation to the problems of actually getting varieties into the market considerable discussion centred upon the difficulties often encountered with registration procedures. There is clearly a need for dialogue between breeders and the respective authorities to ameliorate these problems. In relation to the theme the workshop and first poster session highlighted the requirement for more research into selection criteria and methods of variety evaluation.

The second and third sessions were interrelated in that they considered novel strategies and biotechnology in fodder crop breeding. Topics presented ranged in diversity from the utilization of selfing in breeding programmes through to the use of RFLP's for the mapping of genomes as aids to the selection of superior genotypes. It was apparent that some of these new approaches are closer to practical application than others. Overall a sense of realism prevailed on the time scales necessary before some of the techniques may be routinely applied. This was further emphasised in the poster session devoted to these technologies.

The final session consisted of invited papers considering perspectives in fodder crop breeding. Here the integration of some of the new technologies with the more intractable problems of deciding on breeding objectives for the next century provided a thought provoking close to the conference.

As well as a most comprehensive programme of papers, poster and workshop sessions the organizing committee arranged for visits to the Centre for Plant Breeding Research, the Centre for Variety Research and to the Research Station of Barenburg BV. In addition some most enjoyable entertainment filled the few remaining hours of the programme. We most gratefully acknowledge the support from the many official bodies and companies that contributed to the meeting and to the local committee for their efficient organization of a most successful conference.

M.D. Hayward
President, Eucarpia Fodder Crops Section,
Aberystwyth,
Wales.

December 1990

INTRODUCTION

PLANT BREEDING AND SEED PRODUCTION OF FORAGE CROPS IN THE NETHERLANDS

M. Kamps

President Dutch Agricultural Breeders and Seed Trade Association[1]

Summary

A survey is given of the development of plant breeding in agricultural crops since the rediscovery of the Mendelian laws.

Until about 1950 the breeding of forage crops was hampered by the difficult seed production of new varieties and the severe price competition of seeds, that were harvested on natural pure stands. The present good position of Dutch grass varieties on the markets in many other countries can be attributed to:

- the activities of some pioneer Dutch grass breeders, who started their breeding work already in the early twenties;
- the activities of Dutch research-institutes for breeding research, variety- and seedtesting;
- the Dutch government, that introduced already in 1942 a legal protection for new varieties, thus enabling the private breeding companies to increase their investments in breeding;
- the activities of the seed industry, that was - and still is - prepared to invest the necessary amounts of money in breeding new varieties.

The merging of seed companies into financially strong entities has created a situation, where breeders, seedhouses and farmers can and will take over a part of the responsabilities for routine-testing, thus allowing the governmental institutes to concentrate on the more fundamental aspects of breeding research.

Introduction

Long before the rediscovery of the Mendelean Laws attempts have been made to improve the quality of agricultural crops by selecting the better plants for producing seed for next years' sowing.

In the beginning of the twentieth century several new genetic features were discovered, but their application in practical plant breeding was still limited. It lasted until about 1920 before private persons and companies started practical breeding in agricultural crops. Soon it became clear, that plant breeding was an expensive activity. Often breeders did not get an reasonable renumeration for their work, because other companies could multiply and sell seed of a new variety as soon as it appeared on the market. Already in 1942 the Dutch government took a very wise decision and issued the Plant Breeders Decree; this decree protected the breeders of a new variety against unauthorized propagation of their varieties.

This - in gobal sight - very early possibility for plant breeders protection has been an extremely important instrument to induce Dutch breeders to increase their efforts in plant breeding. As a consequence shortly after the end of World War II the plant breeding activities of Dutch breeders increased tremendously, which gave them a leading position compared with many other countries, where plant breeders rights were enacted only after the conclusion of the Convention of Paris in 1961.

However, since other countries got also a legal protection of new varieties, and also partly due to the compulsory variety testing and seeds certification in the whole EEC, in most crop this initial advantage of our country diminished or disappeared, but it continued to exist for some crops, i.a. for grasses.

By now we are approaching the end of the twentieth century and we are facing the introduction of new technologies in plant breeding. Whether or not genetic engineering will be the panacea that will solve most of the problems in practical agricultural plant breeding, will become clear in the next few decades.

The role of the institutes

We are happy that in a very early stage our government recognised the importance of plant breeding. As a result already in 1912 the Institute for Plant Breeding (IVP) was founded as a part of the Agricultural University in Wageningen. This institute assumed the combined responsability not only for breeding research and teaching but also for testing of varieties and issuing the annual Descriptive List of Varieties.

In the late forties it was decided that the IVP as a part of the University should concentrate on teaching and scientific research, whereas the Foundation for Plant Breeding of Agricultural Crops (SVP) would concentrate on more practically orientated breeding research and the IVRO, later RIVRO on variety testing.

Very recently, the SVP merged with some sister institutes to CPO, whereas the research acitivities of the RIVRO and of the Seed Testing Station were combined in CRZ. These three institutes IVP, SVP and RIVRO have done extremely important work to support the Dutch breeders and to encourage them to breed improved varieties.

Role of the Seeds Industry

In former times the seed houses were predominately seeds merchants often owned by one, or only a few families. With the increasing importance of improved, bred varieties, the market share of the breeders/merchants increased at the cost of the pure merchants. The sharply rising investments in breeding, in seed producing, in sophisticated equipment and in marketing, required a capital structure that was beyond the levels that most family companies could, or were willing to, provide. Consequently an enormous concentration took place and is still continuing. It has to be expected that in the next one or two decades again several companies will disappear or merge and that for each species the market will be dominated by only a few companies.

Forage crops

Coming now to forage plant breeding and forage seed production I have to mention first that grass is the natural ground cover in many parts of the Netherlands. Early plant breeding attempts were conducted since about 1920 by selecting healthy and leafly plants in natural populations.

Until about 1950 the impossibility to produce seed on these selections at prices that could compete with seed that could be harvested abroad on natural (almost) pure stands did not favour further investments in grass breeding. Nevertheless the Dutch forage seeds industry owes many thanks to a few hobby-breeders, who continued their selection work, notwithstanding the poor commercial results and perspectives. They have induced, that already before World War II some official research on varieties of forage and amenity grasses was conducted in our country and that some varieties were recommended in the "Rassenlijst".

They also created a genepool that was very important for the later rapid increase of forage crop breeding. In the fifties it became possible to grow grass seed as an agricultural crop, but still it had to compete with the lower price of foreign origins, that was much less adapted to Wester European growing conditions.

It has been a great help for the breeders and for the farmers in our country that an unique co-operation between the government, grass breeders, grass seed merchants, grass variety research at RIVRO, Certifying Agency NAK, Agricultural Advisory Service and farmers organisations, has created the BG-certification system for forage seeds mixtures. In this system the BG identification may only be given for certain mixtures, provided that they were composed only of officially recommended varieties. The national turnover of these mixtures rose from about 500 tons in the mid-fifties to about 2500 tons in the early seventies and to an average of about 6.000 tons in the late eighties. For the time being it is very difficult to sell other forage mixtures than BG. It is clear, that this result could only be obtained by the full support of all parties involved.

This early support for the use of good mixtures of good grass varieties, strenghtened by the high quality of research on grass varieties at RIVRO, has been an important factor to induce Dutch grass breeders to do their utmost for breeding ever better grass varieties.

Many Dutch good varieties showed also good results in other countries with a moderate climate. Official Lists of Recommended Varieties and reports on variety performance from almost all countries in the world prove that many Dutch grass varieties perform well, even far from home. But buyers of top varieties also want high seed qualities. i.e. a high purity, freedom of noxious weeds and weedgrasses and a high germination. In order to obtain such high quality seed, each of the Dutch seed producing companies has its own growers advisory service that supervises the production from the choice of the fields for grass seed production by the farmer, until the delivery of the harvested seed to the company.

The combination of intensive breeding for better quality, skilled grass seed producing farmers, ample experience and know-how of grass seed production and -processing in the Dutch seed companies has been and still is the backbone for the quality of Dutch grass seed.

Future

It is always difficult to predict what will happen in the future. At this very moment it is more difficult than ever before, because in a rapidly changing world, a decreasing number plant breeding companies of increasing size, is confronted with changing technologies that require increasing budgets for research, whereas public budgets for breeding research are tending to decline and society is demanding varieties that enable a production of agricultural crops with reduced negative effects on our environment.

One point is clear. Grass seed will ever be needed. Consequently at least some companies will survive to satisfy the needs of the world market for grass seed. Which companies this will be, will depend on your decisions in the near future. I whish that this congress will help you to find the right answers for your survival.

1. Present address: N.Z.P., P.O. Box 516, 3800 AM Amersfoort

THEME 1

WHAT HAS BEEN ACHIEVED IN FODDER CROPS BREEDING?

Main papers

ACHIEVMENTS IN FODDER CROPS BREEDING IN NORDIC EUROPE

Jan Sjödin

Svalöf AB, 268 00 Svalöv, Sweden

The forage crop breeding is often unjustly accused of being less effective than e.g. cereal breeding. Indeed it is true that forage crops cultivars are less frequently released than cereal- or rapeseed-cultivars, where a new one is released nearly every year. There are, however, several good reasons for this. Forage crops are mostly perennial implying a longer breeding process. Most species are crossfertilizers often acting at high ploidy levels, both factors complicating the breeding scheme. The seed production is also from the time point of view a tedious process. The seed yield level is often strongly influenced by environmental conditions and it is not unusual that the seed production takes part in another region than the dry matter production.

The most common reason to release a new cultivar is a higher yield capacity than its preceder. Improvements of quality is unusual, at least in Scandinavia. The yield increase can be a direct effect of the selection work but more common in our countries is that the persistence is improved and an indirect increase of yield is obtained. Other improvements are not always easy to prove in figures but can still be essential for the circulation and success of a new cultivar.

One good example of a direct increase of yield capacity is the tetraploid redclover. The 4x varieties grown today in Sweden yields, during the two years after sowing, about 5-10 % more than the diploid standard (Tab 1.) The disadvantage has been the well known fact with the lower seed setting capacity. Today is, however, the level of seed setting acceptable in cultivars for south Sweden and the forage mixtures containing 4x redclovers are the most attractive to the farmers.

Table 1. DM yields of 2x and 4x varieties of redclover in south Sweden.

Year of approval	Variety		Yield (rel figures) I:1	I:2	ΣI	II:1	II:2	ΣII
1968	SV Hermes II	2x	100	100	100	100	100	100
1970	WW Britta	2x	97	107	100	98	111	101
1982	Sv Fanny	4x	103	117	108	106	126	112
1977	WW Sara	4x	103	115	107	100	129	108

Part of the superiority of the tetraploids during later harvesting years probably also can be ascribed to a better persistence. An improvement of this character is the most common for new varieties of most species in northern Scandinavia. It can deal with improvements of disease resistance, winterhardiness or a better adaptation in general to environmental conditions present. Mostly it is a combination of all three factors. Some examples of such progress in Scandinavian plant breeding are given below (Tables 2-6 and Fig 1).

The norwegian meadow fescue variety Salten, released in 1974, (Tab 2) is the result of mass-selection mainly in local populations but also in foreign material. These selections ended up in a synthetic variety based on 14 clones. The practical growing value of Salten is much greater than can be shown in the figures of the table. Today Salten has taken over completely and Löken is taken out of the variety list. Under worse winter conditions Salten is stronger than Löken against both physical and biotic factors.

Table 2. DM yield of two norwegian meadow fescue varieties.

Varieties	Coastal region of sourthern Norway	Remaining parts of Norway
Löken, t/ha	10.3	8.2
Salten, rel	104	102

In Finland nearly all improvements are connected with changes of winterhardiness. One example of this (Tab 3) is the cooksfoot cultivar Haka (1982). Haka showed a higher DM yield than all the other cultivars grown in Finland. The increase was mainly obtained through a better winterhardiness. The introduction of this cultivar gave rise to a more widely spread growing area of cooksfoot in Finland.

Table 3. DM yield of the finnish cooksfoot variety Haka compared to other grown varieties in Finland.

	Variety	Year I	II	III	Winter injury %
1982	Haka, t/ha	8.6	9.7	8.6	8
1976	Fala, rel	90	94	92	+ 4*
1976	Hera, rel	93	94	90	+ 8***
1976	Tammisto, rel	94	94	92	+ 2**
1982	Frode, rel	97	97	99	+ 5**

The importance of persistence is well demonstrated in redclover cultivars developed for south Sweden (Tab 4). The three cultivars Merkur, Hermes and Hermes II are closely related to each other and Pallas contains a great amount of genes from Hermes II. The repeated selections for persistence gave rise to new cultivars with the same yield level in the first year ley, and considerable improvements of the yield level in the second year ley.

Table 4. DM yields of redclover varieties with different persistence in Sweden.

Year of approval	Variety	Yield (rel figures) I:1	I:2	ΣI	II:1	II:2	ΣII
1937	Merkur	97	99	97	91	98	94
1955	Hermes	100	100	100	100	100	100
1968	Hermes II	101	98	100	116	104	112
1990	Pallas	99	97	99	118	108	114

Selections for disease resistance in laboratory tests often show provable results. One example is the redclover cultivar Britta obtained through repeated selections in large number of plants infected with cloverrot (Sclerotinia trifoliorum). The effect of the resistance was demonstrated (Tab 5) in an experiment where frequency of surviving plants in a second year ley was determined, 20-30 % more plants survived in Britta than in the other two varieties. Under practical conditions Britta showed the better resistance through a higher DM yield in second and later year leys.

Table 5. Resistance to Sclerotinia trifolium, cloverrot in redclover varieties tested at Weibullsholm 1972-1977. % surviving plants in second year ley.

	%	rel.
WW Britta	81	100
Sv Hermes	66	81
WW Resistenta	56	69

Another example of successful resistance breeding is the development of Verticillium resistent cultivars in the beginning of the seventies. In the sixties this desease (Verticilium albo atrum) nearly stopped growing of lucerne in south Sweden. Fig 1 shows the rapid progress in laboratory selection for resistance. Such work resulted in the two varieties Sverre and Vertus which in later year leys outyielded the non-resistent cultivar Alfa II (Tab 6).

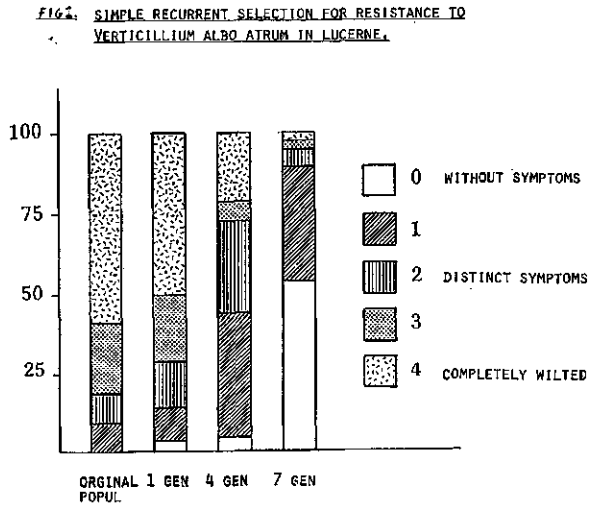


Table 6. DM yields of alfa-alfa. Official trials from south Sweden 1972-1981.

Year of approval	Variety	Yield (rel figures) I	II	III
1967	WW Alfa II	100	100	100
1969	WW Vertus	100	101	105
1974	Sv Sverre	102	101	108

Redclover is a very day-length sensitive species implying that many cultivars are needed to cover such a long country as Sweden. It would therefore be economically important, if possible, to reduce the number of cultivars by extending the cultivation area of a new cultivar. This has been one of the aims of the redclover breeding. The recently released Pallas redclover, is a good example of progress for such a widely adapted type. In table 7 Pallas is compared with the standard in each of the three regions of interest. Pallas is at least as good as and sometimes better than the standards in each region.

In Sweden most leys are composed of one clover species and one or most often more grass species. It is then important that each species in the mixture produce as much as possible when grown together with the other species. That is not always the same as that the cultivar in question is the most high yielding in pure stands. Lorry, a hybrid ryegrass cultivar has a way of less competive growing allowing the other species to grow better during the first year ley. In second and later year leys this results in a higher total yield (Tab 8) for the mixture when Lorry is the ryegrass component.

Table 7. DM yields of Pallas redclover compared with other varieties of current interest in three growing areas in Sweden.

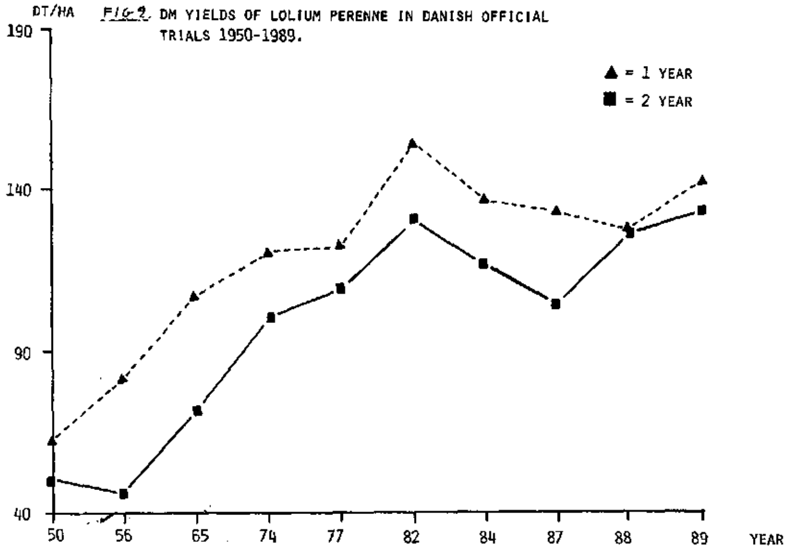
	Yield (rel figures)					
	I:1	I:2	ΣI	II:1	II:2	ΣII
South Götaland						
Sv Bombi	100	100	100	100	100	100
Sv Pallas	102	91	98	108	104	106
Northern Götaland						
Sv Hermes II	100	100	100	100	100	100
Sv Pallas	98	99	99	102	104	102
Svealand						
Sv Kora	100	100	100	100	100	100
Sv Pallas	104	103	104	99	103	100

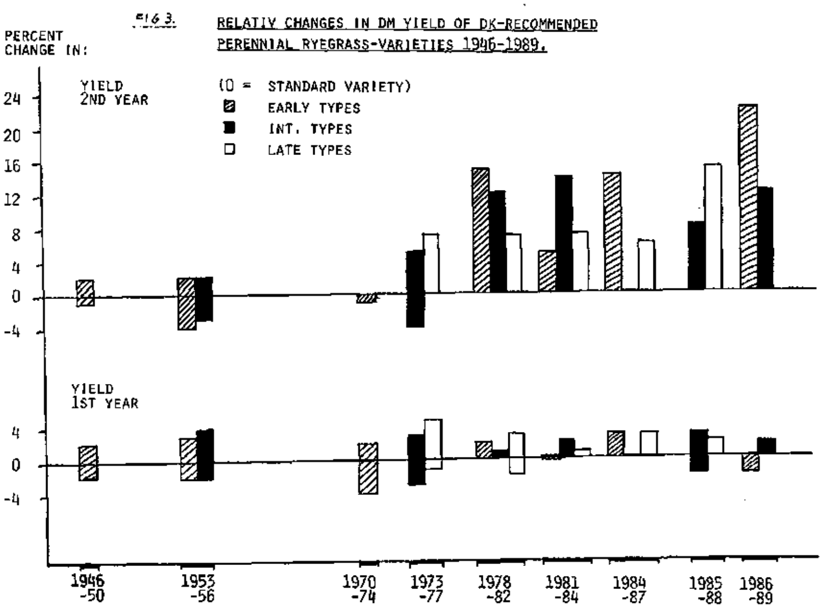
Table 8. DM yield of ryegrass and hybridryegrass in pure stands and in mixtures with clover, timothy and meadow fescue.

Year of approval	Variety	Pure stand		Mixture		
		I	II	I	II	III
1956	Viris, t/ha	10.5	7.8	10.3	8.5	7.6
1984	Tove, rel	108	119	106	109	104
1990	Lorry, rel	110	106	109	110	105

When looking at the development of cultivars of perennial ryegrass (Fig 2) officially tested in Denmark during the last 40 years it can be observed that the increase of yield was considerable during the first 30 years. During some years in the beginning of the eighties the curves levelled out but the yield is increasing again, specifically the second year. These observations are more differentiated in Fig 3 where the relative changes of new cultivars compared to the standards of each testing period are presented for the three types of perennial ryegrass. The figure shows about the same as what was said about the Swedish redclovers. The changes are small in the first year and the main improvements of the new cultivar are obtained the second year. A better Dreschlera resistance is one explanation of these improvements.

During the last 50 years 62 cultivars have been released in Sweden, 18 in Finland and 19 in Norway. In Denmark 83 danish cultivars are included in the present official danish list of cultivars. Altogether it is a considerable number of cultivars even though not all of them have





been a success. The sign of progress of forage cultivars is not always nice relative figures but rather that the farmers like them and buy them.

ACHIEVEMENTS IN FODDER CROPS BREEDING IN MARITIME EUROPE

A.J.P. van Wijk and D. Reheul

D.J. van der Have B.V., Vlijmen, the Netherlands
Rijksstation voor Plantenveredeling, Merelbeke, Belgie

Summary

Results of the government variety testing institutes of Belgium (NDALTP and CLO), the Netherlands (CRZ) and the United Kingdom (NIAB) have been studied for perennial and Italian ryegrass.

For perennial ryegrass it is concluded that newly bred varieties have improved in dry matter yield and persistence compared to old varieties. On average yield increases of 0.5% per year have been achieved over a 25-year period. Winter hardiness, disease resistance and digestibility of new varieties showed varying responses to breeding. The seed yielding ability of new varieties showed no improvement over older varieties.

Improvements in dry matter yield of Italian ryegrass were of the same magnitude as those of perennial ryegrass in the Netherlands and the United Kingdom, while in Belgium no clear improvements were measured.

The major increases in agronomical performance in perennial ryegrass have been obtained with varieties admitted to the official variety lists in the mid-eighties.

Keywords: perennial ryegrass, Italian ryegrass, selection response

Introduction

The Eucarpia Fodder Crops Section held its first meeting in 1967 in Köln- Vogelsang in FR Germany. This first meeting was followed by many more in the various European countries, at which all aspects of fodder crops breeding were discussed: creation and exploitation of genetic variability, breeding objectives and breeding methods. Ideas and research results, exchanged between scientists from universities, governmental institutes and private industry during those meetings, have undoubtedly been incorporated in the research and breeding programmes of the participants, aiming at further progress. The paper presented here attempts to determine what progress has been made over the past 25 years in two fodder crop species: perennial ryegrass (Lolium perenne L.) and Italian ryegrass (L. multiflorum Lam.).

The perennial characteristic and the repeated harvesting of the cross pollinating forage grasses make the determination of possible achievements in breeding difficult. If grass varieties are not properly evaluated, possible progress cannot be expressed. In annual crops with one single harvesting, and even in turf grasses where subjective characteristics play a role, possible steps forward are much easier to measure. Moreover, animal performance will ultimately determine if a new grass variety is an improvement or not.

Next to the question of how to measure progress in breeding forage grasses, comes the question which characteristics might be used to define progress: should dry matter yield be the ultimate characteristic or are characteristics like winter hardiness and disease resistance more important?

In the following paper trial results of government variety testing institutes of Belgium, the Netherlands and United Kindom will be used to determine possible progress.

Belgium

In Figure 1 and 2 data from the national list trials are presented for late perennial ryegrass and Italian ryegrass respectively. These data are collected from the varieties submitted for inclusion in the national variety list from 1963 to 1988. Each mark in the figures reflects the mean value of a variety that completed the total trial cycle.

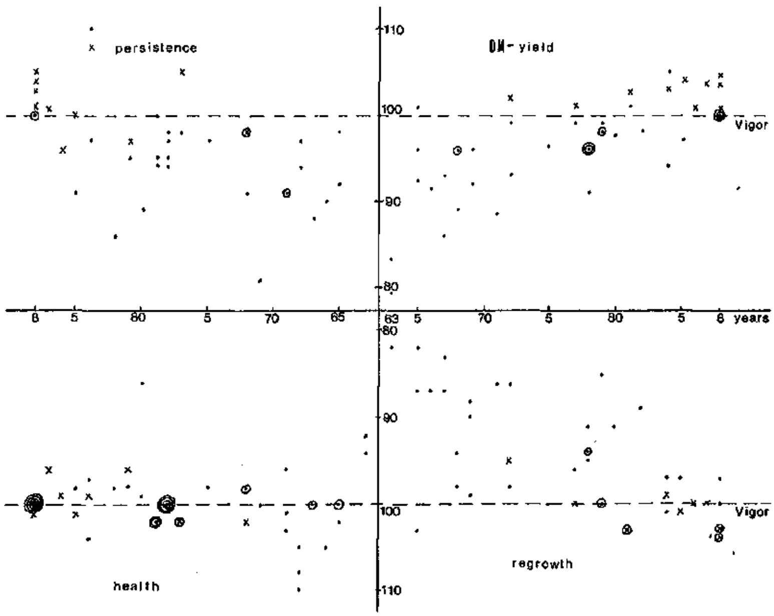


Figure 1: Late perennial ryegrass

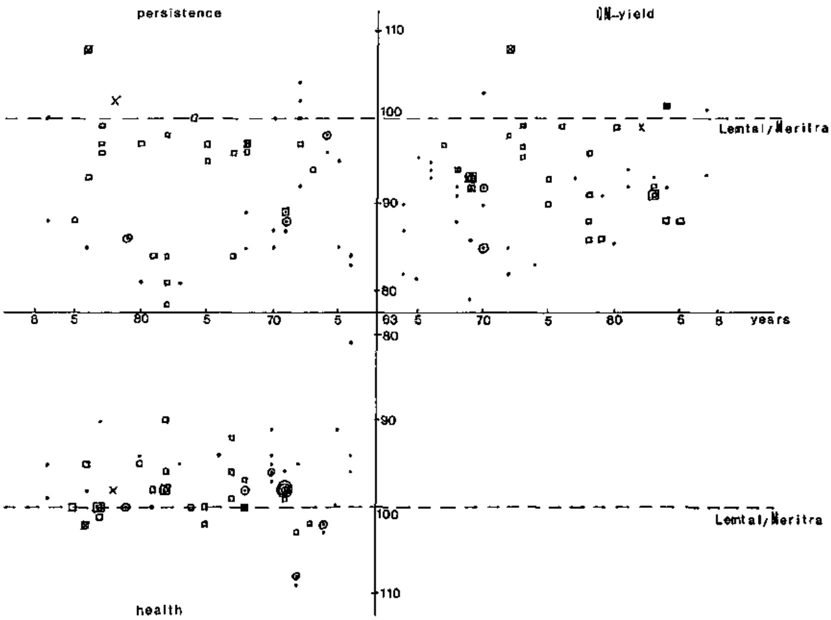


Figure 2: Italian ryegrass

Figure 1 Assessment of late varieties of perennial ryegrass in Belgium
x = listed varieties, o = value occurs more than once

Figure 2 Assessment of varieties of Italian ryegrass in Belgium
x = listed varieties, o = value occurs more than once
◻ = tetraploids

In late perennial ryegrass data are expressed relative to the variety Vigor, which is Belgian bred and is used as main standard in the trials.

From Figure 1 it is clear that the persistence, dry matter yield and regrowing ability of the submitted varieties have steadily increased compared to the variety Vigor. But only from 1980 onwards have new varieties outperformed the standard variety Vigor. Before that time the submitted varieties, which can be assumed to have already passed a pre-screening by the breeders, did not reach the Vigor-levels. As for the health data, no or only minor progress was measured under Belgian conditions.

From Figure 1 it can be deduced that over a 25-year period dry matter yield was increased by 0.5% per year for varieties that were considered good enough to be submitted for national list trials.

For early and intermediate perennial ryegrass similar trends exist. The early heading variety Melino was used as the standard for both the early and intermediate maturity group, resulting in a wider spread of performance data in comparison to the late varieties. Similar to the late varieties, varieties with a higher yielding ability and a better persistence were submitted from 1980 onwards. Health and regrowth were not improved.

The control varieties used in Italian ryegrass are the diploid variety Lemtal and the tetraploid variety Meritra. Both varieties originate from Belgium. As is evident from Figure 2 only one diploid variety and one tetraploid variety were accepted on the national variety list with only marginal yield differences compared to the standard varieties. One of these two showed excellent persistence. No disease improvements were recorded under Belgian conditions.

The Netherlands

In Table 1 the relative dry matter yields of old varieties under grazing and under cutting are presented in relation to the mean yield (=100) of the varieties admitted on the Dutch variety list of 1990. These data have been obtained from trials conducted by the former RIVRO (Rijks Instituut voor Rassenonderzoek), now called CRZ (Centrum voor Rassenonderzoek), since 1978 and were calculated according to the advancing mean (Visscher, pers. comm.).

Table 1. Relative dry matter yields of varieties of perennial ryegrass under grazing (=G) and cutting (=C). Year = First year of admission in Dutch variety list. 100 = Mean yield of varieties inscribed in the Variety List 1990.

		Relative dry matter yield								
Late	Year	G	C	Intermediate	Year	G	C	Early	Year	C
Perma	1939	96	93	Barlenna	1949	94	93	Premo	1955	91
Lamora	1945	97	96	Barstella	1955	93	93	Melino	1960	96
Caprice	1946	89	89	Combi	1958	91	88	Reveille	1965	92
Semperweide	1946	91	89	Spirit	1958	92	83	Cropper	1966	94
Barenza	1949	95	96	Barlatra	1965	90	88			
Compas	1958	93	93	Taptoe	1965	87	90			
Pelo	1962	94	92	Agresso	1969	91	90			
Vigor	1963	96	95	Hubal	1969	94	92			
Mean		94	93			91	90			93

Based on these figures it can be be concluded, that yields of the varieties included in the Dutch variety list have increased by 6 - 10% over yields of varieties admitted since 1939. Comparable to the Belgian situation, a whole range of new varieties have been accepted for the Dutch variety list since the mid-eighties. The last late variety previously admitted was in 1979 and this was the first one after the admittance of Vigor in 1963.

The relative yield of the variety at the time of inclusion in the variety list should be taken into account. When comparing these first figures with later figures some varieties remain constant in relation to the varieties inscribed, while others decline (Table 2). These relative figures are influenced by a change in the varieties inscribed and by a variety times year interaction.

Table 2. Relative dry matter yields of late varieties of perennial ryegrass under grazing and cutting.

Variety	Year of first admission	Weighted mean (4 x grazing + 1 x cutting) 1967	1990
Perma	1939	102	95
Lamora	1945	100	97
Caprice	1946	99	89
Semperweide	1946	102	91
Barenza	1949	101	95
Compas	1958	100	93
Pelo	1962	100	94
Vigor	1963	100	96
Listed varieties		100	100

The increase in dry matter yield of the new varieties was mainly caused by an improvement in persistence. Compared to the older varieties persistence of new varieties is rated 1 - 2 points higher on 1 - 9 scale (9 = best).

In Italian ryegrass yield increases from 1944 onwards varied from 7% - 12 % compared to the varieties listed in 1990. Persistence increased by 1 - 2 points.

United Kingdom

Jordan (1988 and pers. comm.) expressed improvements in ryegrass varieties as the best variety relative to the indigenous landrace varieties (Table 3), based on trials conducted by the NIAB (National Institute of Agricultural Botany) since the 1960's.

The best perennial ryegrass varieties recommended in 1990 produce 20% more yield in the second harvest year than the indigenous varieties. Recent Italian ryegrass varieties show an increase in yield of 25% and 31% in the second harvest year. When calculated over a 25 year period the annual yield increase for both species under UK conditions is 0.5% per year.

In Italian ryegrass a large part of the yield increase was reached in 1968 with the introduction of the variety Lemtal: compared to the local strain Lemtal yielded 120%. Increases thereafter have been slower.

Jordan (1988) also compared persistence, mid-season digestibility values and early spring growth. In perennial ryegrass persistence increased, especially in the early varieties, while in Italian ryegrass sward density of the best present-day variety is double that of S 22. Early spring growth can be found in late heading varieties, indicating the possibility that the two traits can be combined. Mid-season digestibility values are up to 2 units higher in many new perennials and over 1 unit higher in Italian ryegrass compared to that of S 22.

Table 3. Comparison of early, intermediate and late perennial ryegrass and Italian ryegrass. Mean of conservation and simulated grazing yields. 1st line: first harvest year; 2nd line: second harvest year (according to Jordan, 1988 and pers. comm.)

Perennial ryegrass	Old local strain	Early bred variety Diploid	Early bred variety Tetraploid	Top variety 1990 Diploid	Top variety 1990 Tetraploid
Early	Irish commercial	S 24	Barvestra		
	100	105	104	109	109
	100	110	112	119	121
Inter-	Kent indigenous	S 321	Barlatra		
mediate	100	105	103	110	116
	100	102	104	115	120
Late	Kent indigenous	S 23	Petra		
	100	103	102	108	110
	100	103	104	123	120
Mean	100	104	103	109	112
	100	105	107	119	120
Italian	Northern Irish	S 22	Tetila		
ryegrass	100	103	112	127	124
	100	103	110	131	125

Seed yield

How good the vegetative properties of a variety are, the economics of seed production ultimately determines if a variety will be a commercial success. The yield data of commercial seed production fields of perennial ryegrass in the Netherlands (as published by the Agricultural Seeds Produce Board) and in the United Kingdom (as published by the NIAB) do not indicate that the improvement in the vegetative characteristics had a positive correlation with the generative properties. Obviously ryegrass varieties are primarily bred for their vegetative performance; their seed yielding potential is only assessed when the variety comes into commercial seed production.

Discussion

From the data presented for Belgium and the United Kingdom it can be concluded, that the annual increase in dry matter yield of perennial ryegrass through breeding is 0.5% over a 25-year period; the Dutch data indicate a slightly lower increase. For Italian ryegrass in the Netherlands and the United Kingdom comparable advances can be reported. This is not the case for Belgium due to the choice of the control varieties.

However, the data obtained from the official variety testing institutes clearly demonstrate the difficulty in determining the progress made in ryegrass breeding. The results of the various countries can differ to a great extent. These differences might have been partly caused by the evaluation system and the duration of the trialing period (i.e. repeated testing).

In Belgium all submitted varieties were included in the assessment of progress, in the Netherlands old varieties were expressed relative to the mean yield of listed varieties, while in the United Kingdom old varieties were compared to the best current variety. Old varieties have been improved constantly prior to the introduction of Plant Breeders Rights in the mid-sixties, causing a genetic shift and making the comparison between old and new varieties more confounding. Barker and Kalton (1988) used the number of varieties that have been produced as a measure of advancement. However, the quantity of released varieties is not necessarily proportional to the achieved progress.

Dry matter yield is the most obvious tool to assess the level of progress, comprising characteristics that have an influence on variety performance such as persistence, winter hardiness and disease resistance. However, special traits having nothing to do with yield, may be available in a variety making it very suitable for special stress conditions or for improved animal production. The favourable growing conditions for ryegrass in maritime Europe make the expression of such breeding efforts for stress tolerance seldomly visible, while the effect on animal performance is difficult to measure.

It is evident, that a big step forward in variety performance of perennial ryegrass was made in the mid-eighties. These varieties were submitted for variety listing around 1980 and their creation probably started between 1965 and 1970. From then on, breeding apparently intensified, which was caused, according to Lackamp (1988), by the fact that breeders could spend less time on variety maintenance. Around that time variety maintenance through the storing of remnant seed under controlled conditions was introduced and the laborious maintenance of clones and the constant re-creation of the varieties were then abolished. The introduction of Plant Breeders Rights made this necessary as it was no longer permitted to change an existing variety. As a consequence the breeders were left with more time for innovative breeding.

Another reason for the intensification of breeding has been the mechanisation of field trials. Plot drilling machines and specially designed forage harvesters caused an increase in the scale of operations, resulting in the testing of more material and higher selection intensity.

The seed productivity of new varieties has not been increased. During the process of multiplication of breeding material, families may be rejected on the ground of their low seed yielding ability, but apart from this, selection on seed yield as such is hardly made. The reason for this is the complexity of the problem as is very evident from the results of Elgersma (1990).

What are the prospects for further advances in breeding? In diploid perennial ryegrass a high level of performance has been reached and it seems wise to stay in that gene pool, only supplemented with special traits such as disease resistance and winterhardiness. In tetraploid perennial ryegrass the genetic basis is still very narrow and by widening it much progress can be expected. The same holds true for tetraploid Italian ryegrass, but the question can be asked if the diploid basis is wide enough? Big increases in variety performance for this species through breeding cannot be expected.

A worrying development, which could hamper the progress made by breeding, are the rising costs of the official variety testing. Besides this, some countries require pre-trial results from two locations within their countries, before the variety is accepted for another period of 3 - 5 year testing. This delays the release of new varieties and weighs on research budgets enormously, driving out the innovative part of breeding. It is admitted, that the published results of official trial results are a strong marketing tool, but at a certain point it can be queried if it is still worthwile to justify these rising costs. The present system might come to an end and breeders could decide to bypass the present system of government recommendation.

The present system of variety registration is generally accepted and breeders have to live with it. It should be realised, that it is impossible to breed for distinctness with the present evaluation system, but this might change with the development of new techniques. Agronomical good varieties might be stopped from registration by the inadaquacy of the present evaluation system to distinguish different varieties.

References

Barker, R.E. & R.R. Kalton, 1989. Cool-season forage grass breeding: progress, potentials and benefits. In: Contributions from breeding forage and turf grasses, CSSA Spec. Publication 15: 5 - 20.

Elgersma, A., 1990. Genetic, cytological and physiological aspects of seed yield in perennial ryegrass (Lolium perenne L.). Thesis Wageningen. 92 p.

Jordan, W.J., 1988. Improvements in ryegrass breeding due to plant breeding. BGS Research Meeting, Welsh Agr. College, Aberystwyth.

Lackamp, J.W. 1986. Herkomst van de eerste generatie Nederlandse grassen. Centre Genetic Resources, Wageningen, the Netherlands, 18 p.

"WHAT HAS BEEN ACHIEVED IN FODDER CROPS BREEDING?"
CONTINENTAL EUROPE

I. Bócsa

GATE Agricultural Research Institute, Kompolt, Hungary

Summary

The paper gives account about data of 4 East-European countries: Hungary, Czechoslovakia, Rumania and Poland.
The alfalfa is the most important perennial fodder crop in these countries growing on the cultivated area of more than 1 million hectares. 15 varieties were registered in last 10 years, however no one of these exceeded significantly the level of the dry matter production of standard cultivars. At the same time the resistance to Fusarium, Verticillium, Corynebacterium and nematodes showed high breeding progress, which affected the persistence and indirectly the productivity of the varieties. Important results were realized in the breeding of the low content of saponin and seed productivity.
The production of grass species is remarkable in this 4 countries. In last 10 years there were registered 60 varieties among 10 main species in these 4 countries. Breeding progress was seen only in the case of tetraploid Lolium (about 10 %) concerning the dry matter improvement, however the productivity of other species resp. varieties were equal with standard varieties. Some progress were demonstrated in the persistence and winter hardness.
In Hungary were produced an interspecific fertile and productive hybrid between, Bromus inermis and Agropyron repens and in Czechoslovakia were registered a productive hybrid between Lolium multiflorum and Festuca arundinacea which gave dry matter production for 9 % higher than the best standard variety of F. arundinacea.
Keywords: alfalfa, Corynebacterium, Fusarium, persistence, red clover, saponin, tolerance to frequent cutting, Verticillium.

Introduction

In this report I would like to give a general picture about the breeding tasks and results in some countries of previous East-European block, except Bulgaria and Soviet Union. Theses latter two countries, did not give unfortunately account about their work.

Hungary

There are altogether 15 varieties of alfalfa, 7 ones of the red clover and 27 cultivars of 15 grass species on the list of variety catalogue.

Alfalfa breeding

The most important fodder crop is the alfalfa (300.000 ha) among 15 registered varieties, 7 of them were bred in the last decade. The aim of the breeding was accented on the following: resistance to Fusarium and Verticillium, persistence, tolerance to frequent cutting, acknowledge of the irrigation, breeding for the low saponin content, seed production capacity and ability to pasture.
The methods of improvement taken into consideration are: phenotypical recurrent selection and production of synthetic varieties with polycross

tests.
The dry matter production capacity of new varieties does not exceed the standard cultivars but the resistance to wilt-diseases, therefore the persistence, became particularly better (Szarvasi-4, Anna, Alfarez, ÖKI-5). The bearing to frequent cutting of these varieties seemed to be better too at these cultivars.
The variety "Sapko" satisfies special demands, which was registered in 1987. Its saponin content is probably 1/10 part of the standard cultivars. This quality makes it possible to substitute a 40 % of soy bean protein at monogastric animals in form of meal and pellet. At the same time the absence of bitter flavour increases the voluntary intake.
There is another variety with special object: "Róna", which is grown as a pasturetype cultivar with creeping rhizomes. In the mixture with drought resistant grasses (Bromus, Agropyron etc.) it tolerates well the pasturage and it gives also good pastures on dry steppes.
It has to be mentioned that the breeding of the threeway cross alfalfa hybrid cytoplasmic male sterility in Hungary was the first in Europe and second all over the world. However, it was put into circulation only for few years, becaus the heterosis effect did not equalize the cost of complicated seed production.

Red clover breeding

The 7 red clover varieties in Hungary are too many on a small cultivation area which has decreased from 120.000 ha to 25.000 ha in the last decade. Although among the 7 varieties there are 5 tetraploid ones which are produced only for seed export. The cultivation area of red clover is replaced gradually with alfalfa which has more drought resistance and larger ecologic scale.

Grass breeding

In 900.000 ha grassland only 250.000 ha are planted and the rest is extensive permanent grassland (non fertilized, non weed controlled and non irrigated). Grasslands consist of 4-5 grass species and 2-3 legumes. 27 varieties of 15 species are registered and only 6 cultivars were accepted in the last decade. There has been no claim yet to different varieties, only to species. The mostly produced grasses are: Festuca pratensis and F. rubra, Poa pratensis, Bromus inermis and the Agropyron cristatum. The production of intensive grass species as Lolium and Phleum is very insignificant.
The new varieties are different from the old ones in homogenity and they are also more uniform in the distribution of the yield and in the seed production capacity and they have also higher salt tolerance. Objects of breeding are: equal distribution of yield, better drought-, salt-, and cold-resistance, persistence and resistance to leaf-diseases. In these properties, Hungarian germplasms are very valuable and because of their earliness they can avoid the rust infection and they can tolerate drought and the NA_2CO_3 content of the soil and they resist more to late cold than the West-European maritim varieties.
Recently, in Szarvas, a new intergenetic fertile hybrid "Bromag" (between Bromus inermis and Agropyron repens) has been produced, with suitable agronomical values.

Czechoslovakia

Czechoslovakian fodder crop stock consist of 7 alfalfa, 11 red clover (5 diploids and 6 tetraploids) and 33 grass varieties related to 16 species.

Alfalfa breeding

Alfalfa is more important than red clover. Objects of breeding are accented on the resistance to Corynebacteria, Verticillium, Nematode, good persistence and fast regrowth capacity, to high nitrogenase activity, on the tolerance to low PH of the soil and on the large seed production ability as well. The newest alfalfa variety is "Magda" (1986), which was produced with recurrent phenotypical selection. It has a good resistance to wilt-diseases and has significant regrowth ability, higher protein content and very good persistence as well. Therefore, after 3 years of cultivation its yield is more productive than the Palava standard, and it is especially high after the 4^{th} and 5^{th} years of the sowing.
There is also a new cultivar "Zuzana", registered in 1990. It's resistant to leaf-diseases and to wilt and Nematode and it has a higher nitrogenase activity than the standard and it bears the low pH of the soil. Better production ability and resistance, longer persistence than the standard is typical for both varieties, but only 3-4 years after the sowing. The seed production ability is probably 20% higher than in the standard. In 1990 two other cultivars were registered "Lucia" and "Regia", which ones seemed to be better than the standard in seed production.

Red clover

In the breeding of red clover it's very important to take into consideration the resistance to powder-mildew and root-diseases, especially to sclerotinia, and the improvement of seed production capacity. The red clover has also a small cultivation area in Czechoslovakia and has already lost of its importance. The bigger part of 11 varieties was bred a long ago, and the new ones as "Rozal", "Toboz" and "Javorina" are more resistant to above mentioned diseases. The tetraploid Tempus is better in the seed production ability than the standard. There is no significant difference between old and new cultivars concerning the dry matter production.

Grass breeding

In the last 10 years 10 new grass varieties were improved from 6 species. The breeding objects were: dry matter production, persistence, resistance to powder mildew and rust infection, better digestibility of protein, higher content of water soluble hydrocarbons and better palatability. The breeding progress was to be higher in the tetraploid Lolium perenne and Lolium multiflorum ("Odra", "Lolita", "Jivot" and "Tarpan"). The variety "Odra" has more significant digestibility for protein and dry matter. The dry matter production is 10 % higher than in the older diploid varieties. Breeding progresses were found in "Slezanka", Poa pratensis (108 %) in "Lekora" and "Kora", Festuca arundinacea (107 %). There have been no breeding progress in Phleum pratense, Festuca pratensis and Dactylis glomerata for 10 - 15 years.

Nowadays an intergeneric hybrid "Felina" (between Lolium multiflorum and Festuca arundinacea) gave good results in dry matter productivity (109%) compared to "Lekora" Festuca arundinacea, however its protein content was smaller. This one is a festucoid type, "Becva" is a loloid one.

Rumania

In Rumania there have been a lot of activities about the breeding of 24 species of fodder-crop, containing 14 perennials and 10 one-year-old ones.

Of the late ten years 4 varieties of alfalfa, 2 of white-clover and 11 species of grass were registered.

Alfalfa breeding

The main objects of breeding are: production stability, resistance to diseases and abiotic stresses, persistence, higher protein content and ability to seed production.

The breeding progress in the last 10 years was quite modest from the point of view of the increase of dry matter production, so this object was given up. First of all the quality of fodder crop has to be improved in Rumania with the breeding of shorter internodium varieties and which has more leaf mass and somehow differs from the impossible realization of the improvement of leaf/stem ratios. This sort of plants has more resistance to lodging. The best results were reached with the increase of seed production ability, which was carried out with the selection of larger pollen amount. This property was investigated on the field with the help of tripped flowers on a black film. The variability is rather high respect. The selection is based on the relationship between large quantity and the transport of pollen. The main properties of registered alfalfa varieties are the following: "Lutetia": the protein content is 18 % higher than the standard and there is a 4 % increase in the digestibility. "Gloria": Resistant to Fusarium, therefore it has a very good persistence and ability to association with grasses, especially with Dactylis.

The newest cultivar is "Adonis" which is resistant to Fusarium and has an excellent seed productivity as well. In 1984 it reached a record yield, and gave 1224 kg/ha.

Red clover and grass breeding

Since 1977 there has been no new result in the red clover improvement (In 1977 the "Napoca-Tatra" was registered). This plant species is decreasing gradually in the production, because its production area is replaced with alfalfa, which has wider ecologic scale.

In last 10 years 11 varieties of 5 perennial grass species were bred and registered (Festuca pratensis: "Paltar", "Marta", "Mara", Lolium multiflorum: "Tetraiar", "Arina", Dactylis glomerata "Poiana", "Olimp", "Intensiv").

These varieties do not differ significantly from standards in the dry matter production, but their resistance and perennity are on a higher level.

In Rumania permanent grasslands are more characteristic, especially in suitable areas for them as in Carpathians and Subcarpathians area.

Poland

The area of fodder crops includes over 1 million ha of clover and alfalfa and over 3 million ha of perennial grasses. The red clover is more important in Poland than the alfalfa. Red clover share about 60 % of total forage area.

Red clover breeding

Tetraploid red clovers are grown in Poland but diploids are still predominant. Three tetraploid varieties and four diploids have been registered now, and two new populations are in the state trials since, 1986. Varieties NIKE (reg. 1982) and PARKA (1982) are, diploids semi early, and RABA is of late flowering one cut type. ULKA is tetraploid varety. The level of productivity of Polish varieties is as high as European standards. To reach this standard following breeding works have carried on: resistance to Sclerotinia trifoliorum Erikss and Erisiphe martii Lev., improvement of seed productivity, selection high yielding, resistant plants. Due to luck of basis research the progress is slow coming up.

White clover breeding

There are 7 varieties of white clover registered in Poland. RADI (1989) is Ladino type, REMA (1076) and ALDA (1983) are typical Dutch clover, the others: ASTRA (1980), ANDA (1984), ARMENA (1986) and SANTA (1986) are of intermidiate type. The varieties produces good dry matter and protein yield, are winterhardy and persistent. RADI performed very well in clover-grass mixtures. Because of luck of proper machinery seed production is limited.

Alfalfa breeding

Alfalfa is used generally for silage and some part for dehydratation and green fodder. Four Polish varieties and 27 of foreign origin were included on the import list in 1989 in Poland. Import from France, Canada, USA, Hungary covers usually over 80 % of seed demands in Poland. Polish varieties BOJA (1982) TULA (1983) and RADIUS (1988) possessed good winterhardiness and seed setting. The research and breeding programs are directed to improve of: resistance to Verticillium, Pseudopeziza, good seed setting, dry matter and protein yield potentials. Remarkable progress has been achieved increasing seed yield and cold tolerance (cv. RADIUS).

Grass breeding

The main grasses used in Poland are: Festuca arundinacea Schreb, Festuca pratensis Huds, Dactylis glomerata L., Phleum pratense L., Poa Poa pratensis L. , Lolium perenne L. (2n, 4n), Lolium multiflorum Lam (4n), Lolium x boucheanum Kunth. (4n) (R. Oldenburgicum), Lolium multiflorum Lam ssp. gaudini (4n) (R. Westerwoldicum), Arrhenaterum elatius (L), Bromus inermis Leyss, Bromus unioloides H.B.K.

There are valuable new varieties registered in last five years period as: more persistent perennial rye grass, alternative Westerwold rye grass KOGA, ownless french rye grass WIWENA, winterhardy Bromus unioloides BROMA and others. Many of Polish varieties are registered all over

Europe.

The main research on grass improvement is focussed on: resistance (Rhynchosporium orthosporum on orchard grass, Helminthosporium dictoides on meadow fescue, Puccinia coronata on perennial rye grass and meadow fescue), persistancy of perennial rye grass and meadow fescue, diversity in maturity types (early-medium-late), selection of grasses for a yield potentials and improved quality, genetical study (male sterility and heterosis, interspecific crosses).

From this report we can condude, that in last decade the efforts for dry matter breeding especially at alfalfa were unsuccesfull in the above mentioned 4 countries. However a serious progress can be observed in the improvement of special breeding task as the disease resistance, persistance, tolerance to frequent cutting, low saponin content and seed production ability.

It seems that theses breeding objects have to be realised in the future too to serve an indirect increase of the yield.

Acknowledgements

I would like to thank Dr. O. Chloupek, Dr. V. Mrazková and Dr. M. Sevcikova (Csechoslovakia), Dr. P. Varga (Rumania), and Dr. Z. Staszewski (Poland) for their helpful collaboration.

ACHIEVEMENTS IN FODDER CROP BREEDING IN MEDITERRANEAN EUROPE[1]

F. Veronesi

Istituto di Produzioni vegetali, Udine, Italy

Summary

An analysis of the results of breeding for increased dry matter yield (DMY) achieved in Mediterranean Europe in the last 20 years is presented.

Considering data relative to DMY reported in the literature the following results can be drawn:

- Dactylis glomerata: 2% and 4% increases were achieved in France and Italy, respectively;
- Festuca arundinacea: 2% increase in France, 11% in Italy, 7% in Spain;
- Lolium multiflorum: 9% increase in France;
- Lolium perenne: 5% increase both in France and Italy;
- Medicago sativa: 11% increase in France, 6% in Greece, 9% in Italy and 14% in Spain;
- Trifolium pratense: 7% increase in France;
- Trifolium repens: 4% increase in Italy;

It is a general feeling of forage breeders that the limited progress in breeding for DMY is a consequence of the still not well understood gene action on yield in forages. Better methods and techniques together with more researchers involved in forage breeding are needed for exploiting the genetic variability still available.
Keywords: South Europe, dry matter yield, breeding results.

Introduction

The main objective of the present paper is to summarize the results obtained in southern European countries (southern France, Greece, Italy, Portugal and Spain) in breeding for dry matter yield (DMY).

It is well known that the genetic improvements in both forage grasses and legume have been small compared to those obtained in grain crops. The low responses to selection have to be at least partially due to the following reasons:

- the entire plant is of economic value and it is not possible to shunt photosynthetic products to organs of greater economic value such as in grain crops (Hill, 1987);
- in a large majority of forage plants year effects are often confounded with age and stand effects (Hill et al., 1988) and perennial habit increases the time of selection cycle.

Furthermore, most characters concerned with DMY in forages are characterized by low heritability (Taylor, 1987), gene action for yield in forages is not as well understood as it is for grain crops and the plants under selection are often analyzed in conditions (nurseries of spaced plants) different from those typical of the field (Rotili, 1972).

1. Research supported by the Ministero dell'Agricoltura e della Foreste, Italy, Research project "Foraggicoltura prativa", Research Unit at Istituto di Agronomia generale e Coltivazioni erbacee, Sassari, Italy

In this situation, the absence of correlations which has been generally observed between DMY under conditions of artificial selectionand DMY under situations of interplant competition in the field makes selection for yield unreliable (Panella and Lorenzetti, 1968; Ghesquiere et al., 1989).

On the basis of similar considerations, Rotili and Picard (1987) pointed out the importance of studying better methods and techniques for exploiting of the existing variability for yield. It is a general feeling of the researchers that the use of interplant competition during all breeding phases (Rotili, 1984), the greater understanding of tetrasomic inheritance in autopolyploid species, the improvement of breeding methodology, the evaluation in a consistent manner of the dynamic nature of forage accumulation and the better understanding of the effect of natural selection that occurs during seed increase and stand establishment (Veronesi and Lorenzetti, 1983: Hill et al., 1988) may provide important insights into breeding for DMY in the future.

Southern European achievements

Looking at the Plant Breeding Abstracts from 1970 to 1989, 136 out of more than 13,000 references reported under the topics herbage crops, grasses and leguminous forage crops deal with breeding for DMY in southern European countries.

From this information and from that kindly supplied by several European colleagues, mainly gathered from official trials related to varietal registration and evaluation, the following results relative to 20 years of breeding research can be drawn:

1. Grasses (Table 1)

Table 1. Average increases (%) in DMY due to breeding research in grasses.

Countries	Species			
	D. glomerata	F. arundinacea	L. multiflorum	L. perenne
France (South)	2	2	9	5
Italy	4	11	0*	5
Spain		7		

*: 0 = no results obtained with breeding research

1A. Dactylis glomerata L.

In France, the DMY increase due to breeding can be considered not more than 2% (Guy, 1982) while in Italy it ranges from 0% (Paoletti et al., 1988) to almost 8% (Lorenzetti and Falcinelli, 1976).

1B. Festuca arundinacea Schreb.

For this species results similar to those obtained with D. glomerata (+2%) are reported in France (Guy, 1982) while average increases of 11% and 7% have been found in Italy and Spain, respectively (Lorenzetti and Falcinelli, 1976; Rotili et al., 1982; Peredes et al., 1986; Piano and Pusceddu, 1989).

1C. Lolium multiflorum Lam.

Interesting results with DMY increases between 5% (Guy, 1982) and 15%

(Allerit, 1986) have been shown in France while no gains have been achieved in Italy (Paoletti et al., 1988).

1D. Lolium perenne L.

Both in France and in Italy an average 5% increase in DMY can be considered as real (Allerit, 1986; Falcinelli, personal communication).

2. Forage legumes (Table 2)

Table 2. Average increases (%) in DMY due to breeding research in legumes.

Countries	Species		
	M. sativa	T. pratense	T. repens
France (South)	11	7	
Greece	6		
Italy	9	0*	4
Spain	14		

*: 0 = no results obtained with breeding research

2A. Medicago sativa L.

Lucerne, with almost 2,000,000 ha grown in southern Europe, is by far the most important forage crop in this region and, as a consequence, it has been and still is the most studied. In southern France data reported by Guy (1976), Genier (1980) and Angevain (personal communication) show an average increase of 11% in DMY, higher than obtained in northern France. A lower but still distinct increase of 6% is reported in Greece (Vaitsis, 1986) while in Italy an average increase of 9% is shown by the data of Panella (1972), Veronesi et al., 1981), Colombari (1983) and Paoletti et al. (1986). Also in Spain, results reported by the Association de investigation para la mejora de la alfalfa (1978), by Hycka (1983) and Delgado Enguita (1988) show an increase in DMY of 14%.

2B. Trifolium pratense L. and Trifolium repens L.

As far as red clover is concerned, a 7% DMY increase has been obtained in France (Allerit, 1986) while an absence of positive results has been shown in Italy (Paoletti et al., 1988); looking at white clover, in northern Italy Paoletti and Locatelli (1989) indicate 4% as the average difference in DMY between the variety "Espanso" and the local ecotype "Gigante lodigiano".

2C. Others forage legumes

In the last decade in southern Europe increasing interest has been given to annual legumes useful for pasture improvement and land reclamation; annual medics (Prosperi et al., 1987) and Trifolium subterraneum L. (Crespo, 1969; Gonzalez Lopez, 1988; Piano, 1989) deserve particular attention. For subterraneum clover advanced breeder's lines and varieties are already available in Italy, Portugal and Spain but at the moment the DMY increase of these materials when compared to the natural populations is not clearly reported in the literature.

Conclusions

Breeding for DMY in southern Europe has produced stable but low responses to selection. Looking at the single species, it clearly appears that the best results have been achieved with lucerne, which is the forage species most involved in breeding programs. As a consequence, it is possible that selection for forage yield has been difficult and the progress slow not only for the technical reasons reported in the introduction of the present paper, but also due to limitations in the number of researchers involved and to limited funding. As a matter of fact, a lot less public and private concern has been directed to forage crops compared with grain crops.

Furthermore, the results of breeding research have not been fully exploited by the farmers through a correct varietal choice; this is particularly true for southern Europe as reported by Desroches et al. (1984). They indicated that in southern France not more than 50% of the farmers were acquainted with the names of the lucerne varieties they were using while the average value for the whole country was 82%.

An even worse situation is typical of the countries in which seed of forage ecotypes can still be sold; for example in Italy in 1985 seed production for lucerne varieties was only 934.4 t out of 5,133.4 t of the total certified seed produced for this species (CEE, 1986), even if from recent data (CRPA Reggio Emilia, 1990) it can be shown that the use of modern varieties instead of the best adapted Italian ecotypes produces an average increase in net income of about 140 ECU ha^{-1} $year^{-1}$.

Consequently, a greater positive effect of forage breeding on southern European agriculture can derive only from the synergic action of greater use of basic genetic knowledge, significant advances in breeding methodology and cultivar development, increase in research positions devoted to forage breeding, adequate seed production of selected materials and deeper awareness of the farmers.

Acknowledgments

Thanks are due to G. Lemaire, Angers, France; P. Guy, Lusignan, France; M. Angevain and P. Mansat, Montpellier, France; H. Kontsiotou, Larissa, Greece; R. Paoletti and P. Rotili, Lodi, Italy; M. Falcinelli and F. Lorenzetti, Perugia, Italy; M. Tavares de Sousa, Elvas, Portugal; L. Olea, Badajoz, Spain; J. Pinero Andion, Magebondo, Spain and I. Delgado Enguita, Zaragoza, Spain for their valuable information and suggestions on the subject of the present paper. Thanks are also due to M. Traynor, Perugia, Italy, for her help with the English form of the paper.

References

Allerit, R., 1986. Espèces fourragères pérennes: progrés rèalisés depuis 25 ans, appréciés à travers l'expérimentation officielle. Fourrages 107:17-33.

Association de investigation para la mejora de la alfalfa, 1978. Memoria 1978, Zaragoza, Spain.

CEE, 1986. Produzione, esportazione ed importazione nella CEE di sementi di specie per le quali è previsto l'aiuto comunitario. Sementi Elette 32: 21-27.

Colombari, G., 1983. Erba medica: importanza della scelta varietale su quantità e qualità del foraggio. L'Inf. Agrario 39: 25653-25656.

Crespo, D.G., 1969. O trevo subterraneo e o desenvolvimento agro-pecuario em Portugal. Melhoramento 21: 467-488.

CRPA Reggio Emilia, 1990. Varietà foraggere: erba medica. Opuscolo CRPA, Reggio Emilia, Italy, N.4/90.

Delgado Enguita, I., 1988. Evaluation productiva de diferentes tipos de

alfalfas en secano. XXVIII Reunion Cientifica de la Sociedad Espanola para el Estudio de los Pastos, 6-10 June 1988, Jaca (Huesca), Spain.
Desroches, R., P. Gayrand & B. de Ponteves, 1984. Données nouvelles concernant les prairies cultivées. Fourrages 100: 129-160.
Genier, G., 1980. La luzerne: le point sur la sélection. Cultivar 128: 38-39.
Ghesquière, M., M. Angevain & I. Olivieri, 1989. Genotype x density interaction for dry matter yield in alfalfa. Proceedings of the XII Eucarpia Congress, February 27 - March 4, 1989, Gottingen, G.F.R., Book of Poster Abstracts Part I, 10-5.
Gonzalez Lopez, F., 1988. Trebol subterraneo. Agricultura: 40-42.
Guy, P., 1976. La luzerne. INRA Station d'Amélioration des Plantes Fourragères, Lusignan, France.
Guy, P., 1982. La selection des plantes fourrageres. Revista da Sociedade Portuguesa de Pastagens e Forragens 3:21-38.
Hill, R. R. Jr., 1987. Alfalfa. In: W. R. Fehr (Ed.): Principles of cultivar development, Vol.2. Macmillan Publishing Company, New York. p. 11-39.
Hill, R. R. Jr., J. S. Shenk & R. F. Barnes, 1988. Breeding for yield and quality. In: A. A. Hanson, D. K. Barnes & R. R. Hill Jr. (Ed.): Alfalfa and alfalfa improvement. American Society of Agronomy, Madison, USA. p. 809-825.
Hycka, M., 1983. Alfalfa en al secano; variedades. An. Aula Dei 16: 318-328.
Lorenzetti, F. & M. Falcinelli, 1976. Valutazione di specie e varietà per il miglioramento genetico di graminacee foraggere per i pascoli asciutti dell'Italia centrale. Riv. di Agron. 10: 94-101.
Panella, A., 1972. Varietà sintetiche di erba medica di recente costituzione. Sementi Elette 18: 33-36.
Panella, A. & F. Lorenzetti, 1968. Choice of the basic plants for improved lucerne varieties. Proceedings of the V Eucarpia Congress. p. 324-333.
Paoletti, R. & C. Locatelli, 1989. Seed yield, fodder yield and persistence in white clover cvs of different origins. Results of trials conducted at Lodi (irrigated Po valley). Proceedings of the XVI International Grassland Congress, 4-11 October 1989, Nice, France. p. 655-656.
Paoletti, R., T. Maggiore, F. Bozzo, G. Parente, C. Locatelli & M. Romani, 1986. Confronto varietale tra cultivars di erba medica. Nota 1. Risultati di un esperimento collegiale nella pianura settentrionale. 1) Porcellasco (Cr), 2) Lonigo (Vi), 3) Pozzuolo del Friuli (Ud). Annali dell'Istituto Sperimentale per le Colture Foraggere 7: 115-141.
Paoletti, R., C. Locatelli & M. Romani, 1988. Miglioramento genetico delle foraggere da prato. Prova di adattamento e resa di cultivar di graminacee e leguminose foraggere importanti per la pianura padana. Agricoltura e Ricerca 87: 23-40.
Peredes, J., L. Olea & P. Verdasco, 1986. Estudio de variedades de festuca alta para su utilizacion en praderas de regadio del S.O. de Espana. Revista Pastos 16: 151-161.
Piano, E., 1989. Basic aspects, objectives and preliminary results of a subterranean clover improvement program in Sardinia (Italy). Proceedings of the XVI International Grassland Congress, 4-11 October 1989, Nice, France. p. 261-262.
Piano. E. & S. Pusceddu, 1989. Breeding new varieties of tall fescue (Festuca arundinacea Schreb.) adapted to Mediterranean environments. Proceedings of the XVI International Grassland Congress, 4-11 October

1989, Nice, France. p. 317-318.
Prosperi, J. M., M. Angevain & P. Mansat, 1987. Objectifs de sélection des luzérnes annuelles pour la zone méditerranéenne francoise. 5th Network of Mediterranean Pastures, FAO. p. 43-48.
Rotili, P., 1972. Main aspects of a breeding method for lucerne using competitive conditions. Istituto Sperimentale per le Colture Foraggere, Lodi, Italy. p. 149-179.
Rotili, P., 1984. Principles and methods of lucerne breeding work practised at the Institute of Lodi. Proceedings of the Eucarpia Medicago sativa Group, 27-30 August 1984, Brno, Czechoslovakia. p. 297-300.
Rotili, P. & J. Picard, 1987. Introduction of new variability, especially for vigour, into a high level genetic material. Proceedings of the Eucarpia Fodder Crops Section Meeting, 22-24 September 1987, Lusignan, France. p. 91-95.
Rotili, P., G. Gnocchi, L. Zannone & M. Romani, 1982. Festuca arundinacea: results of recurrent selection first cycle carried out with frequent cutting and in conditions of plant interference. Genetica Agraria 36: 187-188.
Taylor, N. L., 1987. Forage legumes. In: W. R. Fehr (Ed.): Principles of cultivar development, Vol. 2. Macmillan Publishing Company, New York. p. 209-248.
Vaitsis, T. A., 1986. Research programme on alfalfa in Greece. EEC Agrimed Workshop on Genetics and Improvement of Alfalfa, 10-11 October 1986, Montpellier, France.
Veronesi, F. & F. Lorenzetti, 1983. Productivity and survival of alfalfa hybrid and inbred plants under competitive conditions. Crop Sci. 23: 577-580.
Veronesi, F., A. Mariani, M. Falcinelli & S. Arcioni, 1981. Risultati preliminari di un ciclo di selezione per la produzione foraggera in erba medica. Riv. di Agron. 15: 45-50.

THEME 1

WHAT HAS BEEN ACHIEVED IN FODDER CROPS BREEDING?

Posters

YIELD PROGRESS IN RYEGRASS SELECTION PROGRAMMES FOCUSSING ON DISEASE RESISTANCE

B.C. Boller

Eidgenössische Forschungsanstalt für landw. Pflanzenbau, FAP Reckenholz, CH-8046 Zürich, Switzerland

Summary

Substantial progress in resistance of Italian ryegrass to crown rust (Puccinia coronata) was made in the breeding programmes at Reckenholz. The results of plot trials sown between 1978 and 1988 with new selections having various degrees of resistance showed that the potential progress in dry matter yield (DMY) was markedly delayed when selecting for rust resistance. This was particularly evident in the diploid Italian ryegrasses. With resistant selections, it took 8 years more to achieve a progress of 5 % in DMY over the check variety TURILO than with suscpetible ones. A similar, but slightly shorter delay was observed in the tetraploid material. The selection work resulted in the release of several new varieties which combined, to an increasing degree, improved resistance to crown rust with higher DMY.
Keywords: Italian ryegrass, crown rust, resistance

Introduction

Resistance to crown rust is one of the major goals in the ryegrass selection programmes conducted during the past 25 years at Reckenholz (Nüesch, 1984). With resistant varieties, a high digestibility and palatability of the forage can be maintained during a heavy rust attack, whereas the quality of susceptible varieties is decreasing. However, selection for disease resistance may lead to a decrease in yield (Nüesch, 1988). This paper analyses to which extent this negative correlation diminished the progress in dry matter yield of Italian ryegrass.

Materials and methods

The results of all annual series of plot trials with diploid (2n) and tetraploid (4n) Italian ryegrasses sown between 1978 and 1988 were analysed. Each serie, composed of at least 9 selections including the check varieties TURILO (2n) or LIPO (4n), was tested at 2 to 3 locations with 3 to 4 replications. Dry matter yields (DMY) measured in the first production year (5 to 6 cuts) were averaged over all locations and expressed as % of the check variety. All rust scores available for a particular serie (2 to 5 observations at 1 to 3 locations) were averaged. The scores ranked from 1 (no disease) to 9 (completely diseased). They were expressed as % of the check variety after subtracting 1 from the average score (i.e. score 1 = 0 %, score of check variety = 100 %). For each serie, the three selections (not including the check variety) with the highest DMY and those with the lowest rust score were determined.

Results and discussion

The gap between the average yield of the 3 highest yielding and the 3 most resistant selections was 1 to 5 % in all series with diploid Italian ryegrasses (Fig. 1) and 1 to 9 % in those with tetraploid material (data not shown). It was not until 1987 that the most resistant diploid selections (having a rust score of less than 50 % of TURILO) exceeded the check variety by more than 5 % in DMY. In comparison, the highest yielding selections exceeded TURILO by 5 % already in 1979. In the tetraploid Italian ryegrasses (data not shown), a slightly shorter delay in yield progress was ovserved: with rust susceptible selections, the yield of LIPO was exceeded by 5 % in 1978, and with resistant ones (rust score less than 50 % of LIPO) in 1985.

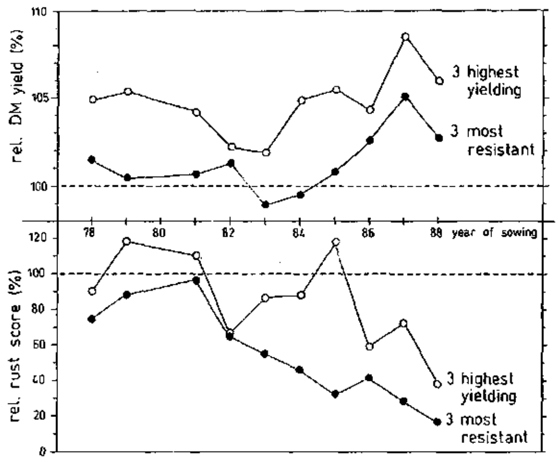


Fig. 1 Progress in yield and rust resistance in diploid Italian ryegrasses (TURILO = 100 %)

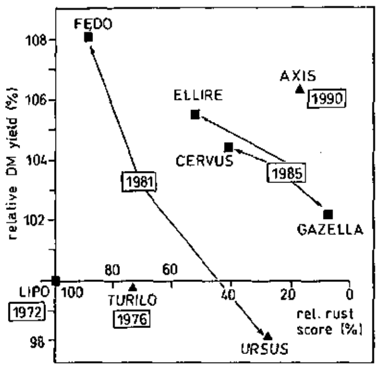


Fig. 2 DM yield and rust scores (LIPO = 100 %) of varieties of Italian ryegrass, with year of acceptance to Swiss "list of recommended grass varieties"

The performances of the 6 new varieties of Italian and hybrid ryegrass, bred at Reckenholz and accepted to the Swiss "list of recommended grass varieties" between 1980 and 1990, show a gradual progress towards improved resistance to crown rust combined with a high yield (Fig. 2).

Although the focus on rust resistance clearly slowed down the progress in DMY, it was possible to achieve resistant varieties with a remarkable yield potential. Apparently, in spite of the negative correlation between yield and resistance, it is possible to successfully select for both characters.

References

Nüesch, B., 1984. Practical implications of development of varieties of red clover and ryegrasses at the Swiss Federal Research Station for Agronomy Zürich-Reckenholz. Rep. Meeting Fodder Crops Section EUCARPIA, Freising-Weihenstephan, 17-20 Sept. 1984, p.33-37.

Nüesch, B., 1988. The effect of selection for disease resistance on vigour of red clover (Trifolium pratense L.) and Italian ryegrass (Lolium multiflorum Lam.). Proc. EUCARPIA Fodder Crops Section, Meeting Szarvas, 5-9 Sept. 1988, p. 44-55.

FORAGE PRODUCTIVITY AND MORPHOGENETIC ADAPTATION AMONG THREE FORAGE GRASS SPECIES UNDER FREQUENT CUTTING

M. Ghesquière, B. Bourgoin, J. Djipdom

Station d'Amélioration des Plantes Fourragères, INRA, 86600 Lusignan, France

Summary

From productivity trials of different forage grass species managed under frequent cutting, the paper suggests to take advantage of genetic variability of morphogenesis components to optimize management of species or cultivars as well as to breed them for a specific management.
Keywords : management adaptation, morphogenesis components, tall fescue, perennial rye-grass, cocksfoot.

Introduction

The yield of forage grasses results from a growth broken off by cut after a more or less long rest period. So, the way and the rate of the growth through morphogenesis (tillering vs elongation of leaves) should be main factors to determine the optimum adaptation of cultivars among a large range of managements. A first experimental approach has therefore consisted in examining the yield of contrasted morphogenesis cultivars managed under very frequent cutting and several cutting heights.

Material and methods

20 forage or turf cultivars (4 cocksfoot - 4 tall fescue - 12 perennial rye grass), sown in swards in April 88 were weekly harvested at 3 cutting heights (3 cm - 5 cm - 8 cm) between the 04/25/89 and 07/04/89, i. e. 11 cuts, under no limiting supplies of nitrogen and water in a 3 replicates criss-cross design. At each cut, canopy height and dry matter yield of the whole design were recorded ; plots of each genotype (3 replicates - 3 cm and 8 cm cutting heights) were also sampled at one cut in the swards to estimate some growth components as tiller density and mean 7 days leaf elongation.

Results

There was a quite strong stability among cuts of the genetic variation for dry matter yield and canopy growth ; genotypic correlations between cuts were always highly significant except at the begining of June where differences of heading date may have leaded to some temporary discrepancies due to the presence of stems in the harvest.

The cutting height has a stronger effect on morphogenetic components, mainly canopy growth and leaf elongation, than genotypic effect but this one only remains significant for yield. Moreover, the lack of significant genotype * cutting height interactions, or low intensity interactions for canopy growth, indicates a quite good phenotypic plasticity of cultivars among cutting heights, even though plasticity and genotypic effect tend to be negatively related.

The joint variations of growth components are well balanced between genotypes and between cutting heights and, when they are combined to yield, it can be seen that genotypic variation is well described according to species or main use (figure 1). But, despite the antagonism between tillering and leaf elongation, the most productive genotypes remain those having the highest leaf elongation although some species particularities imply that other parameters would be involved : tillering through lamina/sheath ratio for tall fescue, growth habit for cocksfoot and some rye-grass cultivars.

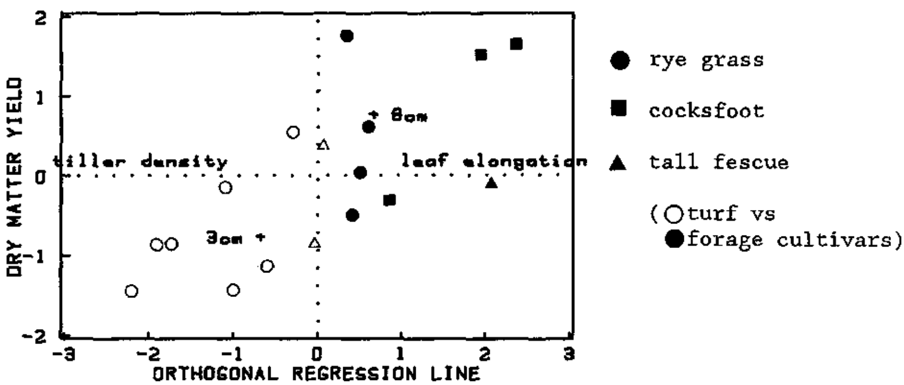


Fig. 1. Relation between morphogenesis and yield under frequent cutting (standardised data). X axis is the orthogonal regression line between leaf elongation and tiller density, correlated with each of them by r = 0.917 and - 0.917 respectively.

Conclusion

The genotypic stability of yield among cutting heights implies that an optimum leaf area index is reached already from 3 cm cutting height and, therefore, that yield in our conditions of frequent cutting during the first year would depend first of all on the vertical distribution of the growth under and above the cutting level. That makes some well-adapted genotypes of cocksfoot or rye-grass emerge which, whatever the cutting height, allocate the growth rather towards elongation than tillering and that, without serious dammage from the plasticity point of view. But one can ask what could be the evolution under such a management and we are developping further observations to assess it. Moreover, as suggested above, it is doubtful that the morphogenesis of every species explains the yield in the same way and we will need also to clarify accurately the most critical ecophysiological growth parameter within each species to consider breeding for management adaptation according to the different aims striven for : productivity, plasticity or persistence ?

TRIFOLIUM MEDIUM L. - POTENTIAL FODDER CROP

S. Góral, M. Sołgała

Plant Breeding and Acclimatization Institute, Radzików, Poland

Summary

Sixteen zigzag clover populations were evaluated under field conditions for morphological characters, seed setting, persistence and winterhardiness.

Evaluated materials displayed high morphological variation. Highly significant differences were found among 16 populations for 8 morphological characters. Seed setting ranged from 19 - 49% whereas the mean value was 36%.

All populations showed a good persistence and winterhardiness (98%).

Keywords: Trifolium medium L., zigzag clover, morphological variation, seed setting.

Introduction

Zigzag clover (Trifolium medium L.) is a long lived perennial occurring in natural sites in Poland (Szafer et al. 1969). It shows a broad adaptation to variable climatic and ecological conditions and tolerance to drought and shading. Their underground rhisomes permit vegetative propagation, and persistancy of plants (Merker 1984). Trifolium medium has higher protein content than T. pratense L. and T. repens L. (Kownacka 1966). The breeding programmes of zigzag clover around the world are concentrated on development of cultivars for grazing and meadow managements. The study reported here discusses morphological variation, seed setting, persistence and winterhardiness of 4 Polish ecotypes and 12 F_1 generations of selected ecotypes in the second year of vegetation. Plants were grown in the field 1 x 1 apart at Radzików, Poland.

Results and discussion

Populations of Trifolium medium L. displayed high morphological variation. Four growth types were distinguished among plants - 1: spreading, 2: ascending, 3: loosely erect, 4: erect. Plants of type one prevailed. Mean number of primary stems per plant was 60.7 (Table 1) whereas together with secondary stems (growing from the rhisomes) this value reached 226 (min. 15 and max. 588 shoots). Variation in seed setting was high as shown by coefficient of variation over 78%. Mean number of heads per plant produced by the primary stems was 352 (Table 1) and 633 for the whole plant. Mean number of secondary stems per plant was 117 and the range of variation of this character was from 0 to 588. The height of plants and the length of rhisomes were less variable as evidenced by lower coefficient of variation 18% and 25% respectively (Table 1). Winterhardiness in all 16 populations was good (98%). Seed setting ranged from 19% to 49% with the mean value 36%.

Table 1. Some characteristics of *Trifolium medium* L.

	Plant	Number per plant			Length
	height	primary stems	secondary stems	heads	of rhiso-mes (cm)
Number of plants	301.0	301.0	292.0	301.0	297.0
Mean	60.7	115.4	117.5	351.9	21.4
Variation coeff. (%)	18.0	47.6	77.3	78.7	24.7

Data presented here for 16 populations of zigzag clover are a part of clover are a part of comprehensive evaluation project of 50 ecotypes collected in natural sites in Poland. Apart from morphological and physiological differentiation, investigated material shows a various level resistance to mildew. Three year trials proved that the persistancy and high production of green mass is an effect of the ability of plants to grow new shoots from developing rhisomes. In mix sowing with grasses, zigzag clover shows higher competitive ability than *T. pratense.*

References

Kownacka, M. 1966. The feeding value of zigzag clover (*Trifolium medium* L.) as a component of mountainous hay.Acta Agraria et Silvestria. VI/2, 93-120.

Merker, A. 1984. Hybrids between *Trifolium medium* and *Trifolium pratense*. Hereditas. 101, 267-268.

Szafer, W. et al. 1969. Rosliny Polskie. PWN, Warszawa.

WHAT HAS BEEN ACHIEVED IN FODDER BREEDING? THE CASE OF DRY MEDITERRANEAN CLIMATES

A. Hadjichristodoulou

Agricultural Research Institute, Nicosia, Cyprus

Introduction

The typical Mediterranean climate, with major portion of the precipitation concentrated in the winter months, has significant variations. In the Western parts of the Mediterranean Sea, annual precipitation is relatively high with adequate precipitation during the spring and autumn months, and very limited in the summer months. However, in the Eastern parts of the Mediterranean Sea (Cyprus, Southern Turkey, Greece, and Italy, the Middle East and North Africa), spring and autumn receive very little precipitation and summers are practically dry.

As a result of these climatic differences, the fodder species grown and management methods applied in the dry Mediterranean areas are different from North-central Europe. For example, permanent pastures with annual or perennial grasses (lolium, festuca) are not successful in the dry Mediterranean climates. Instead, annual dryland crops such as barley, oats and vetch, produce the major quantities of herbage for grazing and hay, as they mature before the onset of the dry season. Contrary to other European regions, there are no sown permanent pastures, because of long (5-6 month) dry late spring, summer and early autumn period.

Results and discussion

Breeding work to improve crops for the dry Mediterranean climates is very limited. Some national Institutions and the international Center for Agricultural Research in Dry Areas (ICARDA), Syria, select varieties and breed fodder crops for these areas.

Barley is used for grazing, hay and straw plus grain production. It is the most adapted crop, giving the highest forage or grain yield in the dry areas. Special forage types have been identified, for hay making (Morocco 628) and for grazing (48 Alger) (Hadjichristodoulou, 1976). A breeding programme at the Cyprus ARI aims at developing hooded types or smooth awned types, to improve the palatability of hay. Genotypes were selected combining both, high forage yield (hay) and grain yield, while other genotypes gave either high yield of hay or grain. Establishment of permanent self-reseeding pastures with medics or clovers has not been successful in the Mediterranean Middle East countries. Barley proved to be the most productive crop in pasture lands, but it requires the self reseeding mechanism (Hadjichristodoulou, 1988). This is being transferred from wild barley, *H. spontaneum*, abundant in the Mediterranean countries. As a result, a successful five year barley pasture has been established in Cyprus.

Oat varieties for hay production have been identified and released for their higher dry matter yield or extended period of hay making (Droushiotis, 1990).

Common vetch varieties giving higher hay yields were selected in Cyprus and ICARDA. Woollypod vetch varieties are evaluated. A species, Vicia amphicarpa, selected by ICARDA in the Middle east and now tested in Cyprus and other countries, produces subterranean seeds and above the ground. This is being studied in several countries as a permanent pasture crop in the dry areas, as it is self-regenenerated, especially through its protected subterranean seeds (ICARDA, 1990).

Medics are being tested by ICARDA as pasture crops in rotation with wheat. Establishment of medic pastures in Cyprus, N. Africa, Jordan and other countries has not been successful, and has very limited application.

Irrigated lucerne or Trifolium alexandrinum (berseem) are grown in limited areas where irrigation water is available. In Cyprus, the lucerne variety Local was the best among many introduced varieties (Droushiotis, 1985).

Conclusion. The progress made in the dry Mediterranean areas through the limited breeding work so far, suggests that it is possible to increase forage production. However due to the different agroclimatic conditions, genetic material developed and practices established in North or Central Europe may not be suitable for the dry Mediterranean climates.

References

Droushiotis, D., 1985. Yield and quality of new lucerne varieties. Miscellaneous Reports No. 20. Agricultural Research Institute, Nicosia.

Droushiotis, D. 1990. Effect of variety, seed rate and cutting stage on forage yield of oats (Avena sativa). Technical Bulletin. Agricultural Research Institute, Nicosia (in press).

Hadjichristodoulou, A., 1976. Effect of genotype and rainfall on yield and quality of forage barley and wheat varieties in a semi-arid region. J. Agric. Sci. Camb., 87: 489-497.

Hadjichristodoulou, A., 1988. The use of Hordeum spontaneum to breed barley for grain and self-regenerating pasture. Cereal Breeding related to Integrated Cereal Production, Pudoc, Wageningen, 195-199.

ICARDA, 1990. Pasture, Forage and Livestock Program - Annual Report for 1989, ICARDA, Aleppo, Syria.

A MULTIVARIATE APPROACH TO GRASS BREEDING

M.O. Humphreys

AFRC Institute of Grassland and Environmental Research, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed, SY23 3EB, U.K.

Summary

Grass is a complex crop and a range of seasonal growth, survival and quality traits must be considered when breeding improved varieties. Multivariate approaches using knowledge of genetic relationships can identify useful genetic resources and ensure efficient selection.
Keywords: principal components, cluster analysis, selection index, economic value.

Introduction

Grass breeding programmes seek to improve a wide range of attributes, including patterns of seasonal growth, persistency factors, such as environmental stress tolerance and resistance to pests and diseases, and quality factors such as the protein and soluble carbohydrate content of cells and the digestibility of cell walls. These broad agronomic characteristics comprise many physiological traits. Thus seasonal growth is affected by flower initiation and soluble carbohydrate content reflects a balance between carbon fixation and utilisation. Genetic variation in these traits is largely manipulated using quantitative methods, although more discrete quantitative trait loci (QTL's) may be identified in future using DNA markers. However, the phenotypic expression of major genes may also depend on a complex genetic background. Therefore, it can be very misleading to consider genes, physiological traits or even broad agronomic characteristics in isolation during breeding programmes. Multivariate quantitative genetic approaches provide efficient methods of assessing basic genetic resources and selection criteria based on the minimum of data obtained as quickly as possible. This should result in highly cost effective breeding programmes.

Results and discussion

Genetic resources

Principal components extracted from genetic correlation matrices may be used in cluster analyses to form groups of populations and determine genetic distances between them so that potentially useful crosses may be identified. In this way 81 ryegrass accessions were assigned to four groups using 24 traits. Considerable heterosis was found in crosses between groups separated by genetic (Mahalonobis) distances of 7.8 and 6.0 but not between groups separated by a distance of 2.5. This suggests that crosses with good potential for breeding can be predicted.

Selection criteria

Genetic correlation matrices can be used to form selection indices. In matrix notation a basic index model can be written as P b = G v where P is a variance/covariance matrix of measured traits and b is a vector of associated weights which can be used to produce individual index scores for selection. If P is known, b may be calculated from knowledge of G, ie the variance/covariance matrix describing genetic relationships between measured traits and characteristics of well defined economic importance whose relative value are indicated in the vector v. The economic value of improvements in characteristics such as dry matter production and feed quality may be found using the approach outlined by Doyle and Elliot (1983). Values of 0.04 £/kg/ha, 0.06 £/kg/ha, 0.04 £/kg/ha, 0.002 £/unit DMD/kg/ha and 4.58 £/unit ground cover (0 - 9 scale) were obtained for spring yield, summer yield, total annual yield, quality and persistence. In a 3 year replicated trial of 250 half sib families derived from crosses between genetically distant populations, economic characters were calculated from the dry matter yield and digestibility of 8 cuts in year 1, 5 cuts in year 2 and a ground cover score in year 3. A selection index approach was used to determine the effect of basing selection on a reduced number of measured traits. Table 1 shows the weights obtained for selection indices containing various subsets of measured traits together with r(TI) values, ie correlations between the desired aggregate genotype comprising economic characteristics and aggregate phenotypes comprising subsets of measured traits. It is evident that a restricted subset of traits can produce an efficient selection response and that, apart from ground cover, year 1 data can provide an effective basis for selection. This is because of strong genetic relationships among measured traits and economic characteristics. A similar approach could be used in future to test the potential of genetic markers and other major gene effects to increase the precision and power of genetic manipulation in plant breeding.

Table 1. Selection index weights and r(TI) values for trait subsets.

Yield year 1 cut 2 kg/ha	0.05	0.06	0.06	0.06
Yield year 1 cut 4 kg/ha	0.06	0.05	0.05	0.06
Yield year 1 cut 5 kg/ha	0.05	0.05		0.05
Yield year 1 cut 6 kg/ha	0.09	0.11	0.10	0.13
DMD year 1 cut 3 %	15.19	17.31	19.38	
DMD year 1 cut 4 %	12.49			
DMD year 1 cut 5 %	5.57			
Cover year 3 0 - 9 scale	4.37	7.59	6.56	
r(TI)	0.82	0.80	0.76	0.74

Reference

Doyle, D.J. & J.G. Elliott, 1983. Putting an economic value on increases in grass production. Grass and Forage Science 38: 169-177.

ABNORMALITIES IN MORPHOLOGICAL STRUCTURE OF THE FLOWER OF ALFALFA (M. SATIVA L.) INBRED LINES

S. Katić

Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia

Summary

Morphological irregularities of the flower have been studied in S_7 inbred lines of alfalfa and their hybrids. Irregularities have been observed in the forming of the pistil, stamens, microspores and macrospores. The irregularities have been present in the inbred lines while their SC hybrids either had no irregularities whatsoever or had them in a considerably smaller percentage.
Keywords:alfalfa inbreds, style, anther, macrospore, microspore.

Introduction

In most alfalfa inbreds, selfing tends to cause reductions in seed set per flower and dry mass per plant. Individual lines suffer general stuntedness, albinism, susceptibility to diseases and the sterility of ovaries and anthers. There seems to be no literature data on the occurrence of morphological abnormalities of the alfalfa flower during inbreeding.

Studying female sterile genotypes, E.T. Bingham and J.H. Pfeiffer (1984) could not find abnormalities of the style and the stigma but found the integument to be underdeveloped. Studying causes of embryo abortiveness in genotypes with good and poor seed set, D.C. Cooper et al. (1937) did not find differences in the structure of ovules but the embryos of the genotypes with poor seed set tended to develop slower and their cells were more vacuolated.

Results and discussion

In our study, abnormalities have been observed on the pistil, anthers, microspores, macrospores and inflorescence tips of alfalfa inbreds.

The pistil

The ovary and the ovules were normally developed but the style and the stigma were missing. The number of ovules was reduced by half. Pistils with missing stigmata and styles were observed in 0.008% of the studied hybrids and 0.01% of the studied inbred lines.

A dwarfed pistil with the usual number of ovules but these were underdeveloped and the whole pistil was smaller by one third from the normal. This phenomenon was observed in 0.03% of the examined ovaries.

Open ovaries were observed in some normally developed pistils and all dwarfed ones. One side of the ovary would not grow together and the ovary appears to be open. The number of ovules is regular but sometimes they are outside the ovary. It seems that the style and the stigma are first to grow together while the ovary starts closing from the bottom, the last point to grow together being the meeting point with the style (Ø.Ø2%).

The style, the stigma and the ovary would not grow together. The style consists of two separate parts positioned one against the other (Ø.ØØ5%).

The stamen

The free stamen is shorter than the other ones. When the free stamen touches the stigma, autogamy is likely to occur.

Microspores (pollen)

The inbreds had an increased number of sterile pollen grains and less pollen than normal.

Macrospores

The ovules, with large micropyles, were elongated in the region of the micropyles and somewhat enlarged with respect to the normal ones (Ø.Ø9%).

Empty ovules were without turgor and appeared aborted. The abortion of ovules progressed from the top of the ovary towards the base, less frequently in the opposite direction (Ø.Ø8%).

The inflorescence tip

As a rule, the inflorescence tip is indeterminate but seldom topped with a flower which usually has an increased number of sepals and petals which are not strictly differentiated. The flower does not have the typical structure but it has two or three pistils grown together.

References

Bingham, E.T. & J.H. Pfeiffer, 1984. Female sterility in alfalfa due to a recessive trait retarding integument development. The Journal of Heredity 75:231-233.

Cooper, D.C., R.A. Brink & H.R. Albrecht, 1937. Embryo mortality in relation to seed formation in alfalfa (M. sativa). American J. of Botany 24:2Ø3-213.

DIFFICULT BREEDING PROBLEM OF FODDER BRASSICAS: TOO HIGH NITRATES CONTENT

Waleria Młyniec

Institute of Plant Genetics, Polish Academy of Sciences, Poznań - Warsaw, Poland

Summary

After self-pollination of two hybrids of fodder Brassicas (with B.c.ssp.pekinensis cv.Granat involved) three types of plants were found (seed, intermediate and fodder) differing in green parts formation and also in the rate of generative reproduction. Investigation on the nitrates content in green parts of seed and fodder group of plants were carried out and revealed great differences. In individual plants differences were even much higher. The results for fodder forms oscilated or exceeded limits from the health of animals point of view, but still genotypically conditioned. That gives the chance for breeding.
Keywords: Brassicas, hybrids, cv.Granat, self-polination, fodder forms, nitrates.

Introduction

Works on genetically conditioned nitrates content in green parts of winter fodder Brassica plants are carried out for some years (Młyniec, Heimann 1985, Młyniec, Blaim, Płoszyński, 1989).

Material and Methods

Hybrids (Balicka, Barcikowska, Młyniec, Zwierzykowska 1980): B.c.ssp.pekinensis cv.Granat x B.o.ssp.acephala cv.Normal, B.n.fodder cv.Bishop x B.c.ssp.pekinensis cv.Granat.

Reproduction of hybrids was carried out by self and open-polination in field experiments. Nitrates content estimation - made by colorimetry method (Brzeziński, COBORU).

Results

Segregation in the result of self-pollination especially, in the progeniec (F_8 - F_{10}) of hybrids is still being observed. Three types of plants were separated in hybrid lines: seed, intermediate and fodder. Mean content of nitrates in green parts of seed and fodder types was estimated. Variations for group of plants were: 0.04 - 0.16 for seed and 0.17 - 0.39% D.M. for fodder types. Very high content was

Present address: Laboratory in Warsaw, Institute of Plant Genetics, ul. Nowy Swiat 72, 00-330 Warsaw, Poland

found in individual fodder type plants, at the very early stage of generative development, at the same lenght of vegetation period.

Discussion and Conclussions

The content of nitrates in green parts of fodder Brassica forms oscilated or was higher than the limit (0.25% D.M.) from the health of animals point of view (Lampeter 1978).

The results obtained proving the genotypical differences in nitrates content in green parts of plants confirm the results obtained for Brassica plants earlier (Młyniec, Heimann 1985, Młyniec, Blaim, Płoszyński 1989) and for other plants like Avena sativa L. (Wojcieska, Wolska, Król 1981) and Lactuca sativa L. (Reinink, Groenwold, Boosma 1987, Reinink, Groenwold 1987).

The results of present work indicate that the genotypical differences in nitrates content are highly combined with the physiological aspects of nitrogen metabolism and activity of nitrate reductase enzyme.

The work will be carried out further on.

References

Balicka M., B. Barcikowska, W. Młyniec, E. Zwierzykowska, 1980. Synthesis of new genotypes within the genus Brassica. Cruciferae Newsletter 5: 28.

Lampeter., 1987. EUCARPIA. Report of the Fodder Crops Section. Radzików, Poland, 4-9, September: 5-29.

Młyniec W., S. Heimann, 1985. EUCARPIA. Report of the Fodder Crops Section. Svalöf, Sweden, 16-19 September: 178-184.

Młyniec W., H. Blaim, M. Płoszyński, 1989. Nitrates content in green matter of some Brassica fodder forms, the level of nitrogen fertilization and the time of harvest. Cruciferae Newsletter (in press).

Reinink K., R. Groenwold, A. Bootsma, 1987. Genotypical differences in Lactuca sativa and related species and correlation with dry matter content. Euphytica 36: 11-18.

Reinink K., R. Groenwold, 1987. The inheritance of nitrate content in lettuce (Lactuce sativa L.) Euphytica 36: 733-744.

Wojcieska U., E. Wolska, M. Król, 1981. Wielkość i struktura plonu oraz aktywność reduktazy azotanowej kilku odmian owsa w zależności od wielkości dawki azotu. Pamiętnik Puławski 75: 31-43.

BREEDING OF TETRAPLOID FESTULOLIUM FODDER GRASSES WITH DIFFERENT MATURITY

K. Netzband

Institute of Fodder Production Paulinenaue, Germany D. R.

Summary

Since 1971 in the Institute of Fodder Production Paulinenaue has been working on the production and breeding of x Festulolium hybrids. 1986 the first medium early allotetraploid variety 'Paulita' (Festuca pratensis x Lolium multiflorum) was registered. This variety outyielded varieties of Festuca pratensis and Lolium perenne by 10 ... 20 % dry matter in three using years in state trials. Seed production enterprises yielded 6 ... 9 dt/ha seeds. Forage quality is nearly intermediate to the crossing parents. The stability of the chromosome numbers and morphological and agronomical performance characteristics was good many subsequent generations. Further early to medium late Festulolium strains are in state trials.
Keywords: x Festulolium, breeding, yield, quality, stability.

Introduction

Hybrids from crosses of Festuca- and Lolium species (x Festulolium) are well known for their good vitality many decades ago. This hybrids own a combination of performance characteristics, above all vegetative, which are rarely to find within one of the parent species. Most important breeding aims are dry matter and seed yields, persistence and some quality characters. In this paper ist reported on the results of breeding and on some characteristics of the breeding products in the Institute of Fodder Production Paulinenaue.

Results and discussion

After some generations selection of fertility, good vegetative growth and persistence at least two winters under single plant conditions, favourable plants were cloned. With the seed of this clones onelocated trials were carried out over three using years. Best progenies were tested in state trials. Table 1 shows the results of such trial on 9 sites in the whole country.

Table 1. Dry matter yields (1987 - 89)

Using year	F. p. 'Benfesta' dt/ha	%	L. p. 'Alex' dt/ha	%	Festulolium 'Paulita' dt/ha	%	10/83 dt/ha	%	13/83 dt/ha	%	GD 0,1 dt/ha	%
1.	172,2	100	182,5	106	206,5	120	200,5	116	196,6	114	8,9	5,2
2.	103,8	100	100,9	97	117,7	113	115,0	111	115,9	112	8,1	7,8
3.	98,0	100	100,6	103	113,4	116	104,6	107	106,9	109	8,2	7,2
$\bar{x}$	124,7	100	128,0	103	145,8	117	140,0	112	139,8	112		

F. p. = Festuca pratensis, L. p. = Lolium perenne

In all using years there is a clear superiority to the varieties of Festuca pratensis and Lolium perenne. The best dry matter yields achieved the medium early variety 'Paulita', the medium late strains FLB 10/83 and 13/83 showed something lower yields, but this is known in other grass species also. In 1989 the seed producing enterprises on 1 200 ha yielded in the average 6 dt/ha, good enterprises till to 9 dt/ha. Some chemical compounds, that are of importance for the quality of the forage are given in Table 2.

Table 2. Some chemical compounds and in vitro digestibility of Lolium perenne, Festuca pratensis and Festulolium strains

1. Cut	Crude fibre g/kg DM	Crude protein g/kg DM	Water soluble carbohydrates g/kg DM	Enzyme soluble org. matter %
L. p. 'Alex'	282	134	184	68
F. p. 24/79	319	138	138	61
FLB 10/83	305	115	199	66
FLB 13/83	281	115	204	72
2. Cut				
L. p. 'Alex'	245	163	180	74
F. p. 24/79	223	167	137	72
FLB 10/83	261	128	210	73
FLB 13/83	266	129	208	74

Remarkable is the high content of water soluble carbohydrates. The crude fibre content of the hybrids is in some cases lightly higher than that of L. perenne, while the in vitro digestibility is identical. It is a possible interpretation, that the composition of the crude fibre is different.

One of the most important questions of breeding hybrids is the stability of this breeding products. Zimmermann (1988) found, that the chromosome number of the variety 'Paulita' was highly stabile over 6 generations with an average of 27,9 and 63 % euploids. These results are largely similar to those with tetraploid L. perenne or L. hybridum. Nearly all aneuploids had only one chromosome more or less. There was the tendence, that euploid gametes and plants have a better competitive ability. In a trial for examination the performance stability of 4 generations of the variety there were no significant differences in the dry matter yields over three using years between the generations (Netzband, 1988). So it can assumed, that the stability of this x Festulolium variety is sufficient to preserve the typical variety characteristies in the seed multiplication.

References

Netzband, K., 1988. Methoden, Probleme und Ergebnisse bei der Züchtung leistungsfähiger Futtergräser durch Bastardierung von Wiesenschwingel (Festuca pratensis Huds.) und Welschem Weidelgras (Lolium multiflorum Lam.). In: H. Kaltofen (Ed.): Züchtung und Anbau von Wiesenschweidel. AdL der DDR, Berlin. S. 8-20.

Zimmermann, J., 1988. Untersuchungen des Einflusses der chromosomalen Stabilität auf Ertrags- und Qualitätsmerkmale in der Generationsfolge von Festulolium-Bastardgräsern. AdL der DDR, Berlin, Diss. A.

THE USE OF FREQUENCY ESTIMATES IN STUDYING SWARD STRUCTURE

J.H. Neuteboom, E.A. Lantinga and E.N. van Loo

Department of Field Crops and Grassland Science, Agricultural University, Wageningen.

Summary

Absence frequency estimates of rooted perennial ryegrass tiller bases from sampling quadrats of different sizes are recommended for characterizing the open space structure of perennial ryegrass swards.

- Absence frequencies of rooted tiller bases from quadrats are frequencies of distances from observation points to nearest plants.
- Converted to percentages, absence frequencies of plants from quadrats are the *cover percentages* of points with distances to nearest plants of at least the radius of the quadrats.

Absence frequency estimates are very useful for the evaluation of the persistence of grass species and cultivars in swards. Absence frequency data could also be used directly in simulation models of grassland production for calculating the unproductive area of a sward. For that purpose a critical nearest plant distance from an observation point has to be defined that may not be exeeded for getting maximum production.

A more detailed paper has been submitted under the same title to *Grass and Forage Science*.

GROUPING GALICIAN INDIGENOUS POPULATIONS OF <u>LOLIUM PERENNE</u> CONSIDERING POPULATION X LOCATION INTERACTION

J.A. Oliveira, G. Charmet*

Centro de Investigaciones Ágrarias de Mabegondo, La Coruña, Spain
*INRA, Clermont Ferrand, France

Summary

A sample of 21 indigenous ryegrass populations from Galicia was evaluated in 3 locations (one in Spain and two in France). Genotype x environment interactions make a problem for the use of these populations in plant breeding. To resolve this problem, we used structuration of interaction by automatic classification. Structuration of interaction by ascendant hierarchical clustering leads to a partition into 6 groups, 4 of them showing a particular advantage in each location.
Keywords: perennial ryegrass, genotype x environment interaction, hierarchical clustering.

Introduction

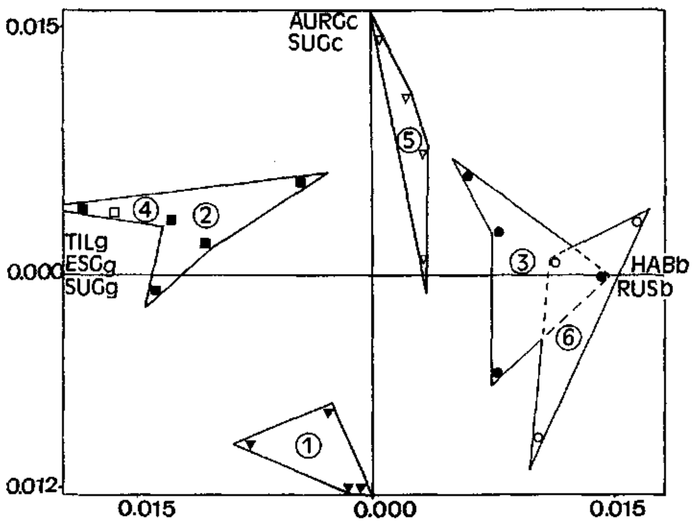
Plant breeders aim to find genotypes which behave well in diferent environments. To this effect they carry out multi-site trials on the range of environments where these genotypes will be used. At the starting point of a forage breeding programme for the Atlantic regions of Spain we collected indigenous populations of perennial ryegrass in Galicia. This collection was firstly evaluated in Galicia, then in two locations in France. The aim of this study is to identify groups of populations which show a particular profile in each location or on the contrary, to obtain stable populations over all locations for common use in Spain and France.

Material and methods

21 seed samples of galician indigenous populations of perennial ryegrass were studied as spaced plants in three locations: Mabegondo is on the Atlantic coast of Galicia (North West of Spain), Clermont-Ferrand is in inland plain with a semi-continental climate and Bourg-lastic is in the wet hill of the Massif Central in France. Each population was represented by 6 complete randomized blocks with 10 plants per block. Seven traits of agronomic value were recorded on a 1 to 9 visual scale: tillering, growth habit, crown rust susceptibility, aftermath heading, early spring-summer and autumn vigour. After variance analysis showing that population and population x location effects, were significant, these efects were submitted to a principal component analysis. In order to give, the same weight to each variate in each location, we used a reduction procedure. A hierarchical clustering from the first 6 principal components was run and a set of partitions was made. Group number were assigned to reduced data for each population and partition, was assessed by the following analysis of variance: y= Mu+Lo+Lo/bl+Gr+Po/Gr+GrxLo+Po/GrxLo+Error, where: Lo=location, Gr= group and Po=population. The significance of GrxLo tested against Po/Gr xLo was used to choose a partition.

Results and discussion

Location x population interaction appears to be highly significant and all traits were considered for interaction study. Total variance explained by between-clusters variance percentage (63,5%), and signification of Gr x Lo tested against Po/Gr xLo suggest a partition in 6 groups. Clusters 1 and 5 are interesting for french conditions and clusters 2 and 4 seems to be interesting in Galicia. Figure 1 shows the position of 6 clusters on the plan of the principal components. The 1st principal component is negatively correlated with the agronomic traits noted in Mabegondo. Axis 2 can be considered as illustrative of french conditions. In this study we suggest to use in selection the interactive populations and with a good agronomic performance for many traits in each location.



TILg = Tillering observed in Galicia; ESGg = Early spring growth observed in Galicia; SUGg= Summer growth observed in Galicia; HABb= Growth habit observed in Bourg-lastic; RUSb=Crown rust susceptibility observed in Bourg-lastic; SUGc= Summer growth observed in Clermont-Ferrand; AURGc=Autumn regrowth observed in Clermont-Ferrand.

Fig.1. Graphic of the 6 clusters on the first two principal components.

References

Denis, J.B., 1979. Structuration de l'interaction. Biom. Praxim. 19:15-34.

Ghaderi, A., E.H. Everson & C.E. Cress, 1980. Classification of environments and genotypes in wheat. Crop Science.20:707-710.

Westcott, B., 1986. Some methods of analysing genotype-environment interaction. Heredity. 56:243-253.

SELECTION OF MEDITERRANEAN TYPE TALL FESCUE FOR WINTER YIELD AND PERSISTENCE

E. Piano

Istituto Sperimentale per le Colture Foraggere, Lodi, Italy

Summary

Summer survival proved to be a distinct attribute of the Mediterranean populations used as a base material for the selection program. Wide variation for winter yield occurred among and within populations, the former being more important than the latter. Joint selection for high winter and total yield was very effective. A diallel analysis indicated that the relative importance of GCA and SCA effects, as expressed by their ratio, varied among seasonal yields.
Keywords: *Festuca arundinacea* Schreb; summer survival; winter yield; selection; GCA; SCA.

Introduction

The use of tall fescue (*Festuca arundinacea* Schreb) and other perennial grasses in the Mediterranean region has been limited by the poor adaptation of the commercial cultivars to the climatic conditions of the area. The ability to survive the summer drought and to grow adequately in winter are primary objectives of selection programs aimed at developing adapted varieties (Piano and Pusceddu, 1989a). Some results related to these aspects of plant selection, with particular reference to winter yield, are presented in this paper.

Materials and methods

Natural populations from Sardinia (Italy) were evaluated for summer survival and yield potentiality under a sub-arid Mediterranean climate, and 300 genotypes belonging to 17 populations were prelimanarily chosen. A further evaluation concerned also populations collected in Sicily (Italy). Subsequently, polycross progeny tests were made involving the whole group of 300 parentals and minor numbers of genotypes selected later from that group because of their outstanding winter and total yield. A diallel cross was also performed with 20 genotypes to evaluate GCA and SCA effects. The diallel progenies were evaluated through 13 cuts.

Results and discussion

The use of native populations as a base genetic material for the selection program proved to fulfil the requirements for plant adaptation to the peculiar Mediterranean environmental constraints. The Sardinian ecotypes showed a virtually complete summer survival, and so did later also the populations from Sicily. On the contrary, the persistence of a range of commercial varieties did not exceed 12%, after 3 years characterized by severe summer drought. Significant variation for winter yield occurred among the 300 genotypes evaluated as polycross progenies. As resulted by the estimates of the components of variance, the differences among populations (provenances) were more important than the differences among genotypes within populations in originating this variation. The preliminary choice of outstanding populations is therefore of key importance for the success of the selection program. There was no relationship between heading time and winter yield.

Table 1. Coefficients of correlation (r) among seasonal and total yields in the polycross progenies of selected genotypes.

	1	2	3	4	5
1 Autumn	1	0.84 (§)	0.83	0.85	0.93
2 Winter		1	0.89	0.76	0.95
3 Spring			1	0.80	0.97
4 Late Summer-Autumn (§§)				1	0.88
5 Total					1

(§) all coefficients are significant at $P < 0.01$; (§§) when irrigation in late summer was provided;

The final selection for both high winter and total yield was very effective and resulted in a set of well balanced genotypes in which seasonal and total yields were highly correlated (Table 1). This was facilitated by the fact that the variation in winter yield was largely a reflection of the variation in plant vigour and global yield potentiality rather than the variation in seasonal production patterns. The results of the diallel analysis indicated the significance of both GCA and SCA effects for winter and other seasonal yields (Table 2). As exemplified by the ratios GCA/SCA the predominance of additive over nonadditive effects seemed to be higher for winter than for residual annual yield.

The selection program has already yielded "Tanit", a synthetic variety with distinct persistence and winter yield (Piano and Pusceddu, 1989b).

Table 2. Analysis of variance for GCA and SCA for winter and residual annual yield evaluated for two years.

Source of variation	DF	Mean squares			
		Winter 1	Residual 1	Winter 2	Residual 2
GCA	19	14117**	125841**	39388**	63449**
SCA	170	618**	7560**	2543**	8388**
Error	567	86	1163	193	1069
GCA/SCA		22.8	16.6	15.5	7.4

** significant at $P < 0{,}01$

References

Piano, E. & Pusceddu S., 1989a. Breeding new varieties of tall fescue (Festuca arundinacea Schreb.) adapted to Mediterranean environments. Proc. XVI Int. Grassl. Cong., Nice, France, 317-318.

Piano & Pusceddu, 1989b. Tanit: una nuova varietà di Festuca arundinacea per l'ambiente mediterraneo. Atti 33° Convegno SIGA, Alghero, p. 81-82.

EFFECT OF SELECTION FOR RESISTANCE OF LOLIUM PERENNE L. TO DISEASES

Pronczuk S., Pronczuk M., Sybilska A.

Plant Breeding and Acclimatization Institute,Radzikow,Poland

Summary

A few selection cycles for resistance of Lolium perenne L. to Microdochium nivale(Fr) Samuels Hallett (Fusarium nivale) and Xanthomonas campestris pv. graminis (Egli, Goto) Dye were performed using laboratory and field screening methods. The obtained plant synthetics (early, medium, late) were tested throughout three years of field utilization. It was found that cyclic selection can improve the yielding and persistancy of early types of Lolium perenne.
Keywords: selection, Microdochium nivale, Xanthomonas campestris pv. graminis, yielding, synthetics: early, medium, late, Lolium perenne.

Introduction

Lolium perenne L.is a low persistent grass in Poland. Sufficient green mass production often takes place only 1-2 years after sowing. The role of severe climate and pathogens interaction is still debated (Tab.1).

Table 1. Correlation coefficients between field yield and lab. tests obtained from breeding materials of Lolium perenne (Pronczuk, Zagdanska 1988 unpublished)

	Field yield			Lab. test	
	1984 mild winter	1985 severe winter	1986 mild winter	M.nivale cold chamber method	Frost Fuller,Eagles method
	1	2	3	4	5
1	1.000				
2	0.253	1.000			
3	0.791**	0.386	1.000		
4	0.836**	0.098	0.892**	1.000	
5	0.386	0.773	-0.014	-0.200	1.000

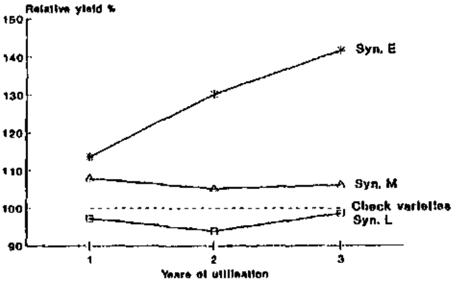
** significant for a = 0.01

It was found that Microdochium nivale(Fr) Samuels Hallett (Fusarium nivale) and Xanthomonas campestris pv. graminis (Egli, Goto) Dye can strongly damage Lolium perenne in Poland (Pronczuk 1987, 1988).
The aim of this investigation was to improve the yielding of Lolium perenne by cyclic selection against M.nivale and X.c.pv.graminis.

Materials and methods

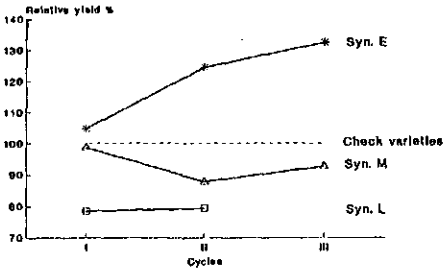
The selection was done in the period 1977-1990. The initial plant material was a collection of 246 clones selected for general purposes from varieties and ecotypes. During three cycles of selection 1047 crosses were conducted. The selection of F_1 plants was done for M.nivale by using the "cold chamber" method (Cormack, Lebeau 1959, Pronczuk 1987).The 83760 plants were checked in "cold chamber".In autumn the plant seedlings were moved from the chamber for winter post tests. In the summer field selection for resistance to X.c. pv. graminis was conducted by method Egli, Goto, Schmidt 1975, Pronczuk, Schollenberger 1988. The best plants after polycross test were taken to synthetics. The synthetics Early,Medium,Late evaluated for fresh mass (f.m.) production at four locations and compared to common check varieties for a three years period. The test for efficiency of three cycle selections was done at Radzikow Exper. Station in central Poland for a three years period.

Results



$LSD_{0.05}$ 3.3% 9.8% 14.2%

Fig.1. Relative yield of new synthetics (Syn) after double selection (average 4 location test). Real yield f.m. of check varieties t/ha in years: Naki 70.7 32.9 26.9, Arka 82.0 40.0 41.9, Aragona 87.9 48.7 32.7 respectively.



$LSD_{0.05}$ - 14.9% (E), 11.9% (M)

Fig.2. Relative yield of synthetics after I, II and III cycles of selection. Real yield f.m. of check varieties t/ha (average 3 years): Naki 40.6, Arka 52.2.

Conclusion

- Selection for resistance to Microdochium nivale and Xanthomonas campestris pv. graminis can improve the yielding of some types of Lolium perenne. In Poland, selection of early type of plants seems to be succesfull.
- M.nivale, X.c.pv.graminis and frost resistance laboratory tests can be usefull for breeding of Lolium perenne in "marginal zone" regions.

References

Cormack M.W.,Lebeau J.B., 1959. Snow mold infection of alfa-alfa, grasses and winter wheat by several fungi unter artificial conditions. Can. J. Bot. 37:685-693.

Egli T., Goto M., Schmidt D., 1975. Bacterial wilt a new forage grass disease. Phytopath. Zeit. 82:111-121.

Pronczuk M., Pronczuk S., 1987. Usefulness of "cold chamber" method for the assessment of resistance of ryegrass to Fusarium nivale (Fr)Ces. Biul. IHAR 162:27-32.

Pronczuk M., Pronczuk S., Schollenberger M., 1988. Effect of fungal and bacterial diseases on the perennial ryegrass stability. Zesz. Prob. Post. Nauk Rol. 366:149-154.

BREEDING WINTERHARDY RYEGRASS SYNTHETICS

Dirk Reheul

Rijksstation voor Plantenveredeling, Burg. Van Gansberghelaan 109, 9820 Merelbeke, België

Summary

The performance of 7 ryegrass synthetics (2 perennial, 5 italian) is presented. All synthetics were constructed with components that survived undamaged the hard winters of 1985-1986 and 1986-1987.

Introduction

After the severe winters of 1985-1986 and 1986-1987, 7 ryegrass synthetics were composed. Selection criteria, ranked in decreasing importance, were :
- winterhardiness
- excellent growth and regrowth
- good crown rust resistance.

On winterhardiness absolutely no exceptions were tolerated, but some very vigourous plants with a moderate level for rust resistance were selected. All candivars were tested in two yield trials. Both trials with perennial ryegrass were sown in the spring of 1988. Figures include 3 growing seasons : 1988, 1989, 1990.
Trial 1 with italian ryegrass was sown in the autumn of 1988 : data of 1989 and 1990 are given. Trial 2 was sown in the autumn of 1989.

Results

See table on p. 2.

Conclusions

1. According to the data it seems possible to breed high yielding synthetics composed with winterhardy plants.
2. The moderate figures for the digestibility - particularly in the diploid italians - are a surprise, since all the synthetics are far more leafy than Lemtal.
3. During the dry summers of 1989 and 1990 the swards of the presented synthetics stayed remarkably dense and green in comparaison to other varieties, pointing to a certain relation between winter- and drought-tolerance.
4. The wintertolerance has not been tested yet in situ owing to the mild winters of 1988-1989 and 1989-1990. However the survival rate of the late perennial was significantly higher than Vigor in a series of artificial frosttests.
5. Synthetics 1, 2, 3, 4 and 7 will figure in the Belgian National List Trials from 1990 on.

Performance of 7 ryegrass synthetics constructed with winterhardy components

Species	Synthetic	Number of components	Trial	Standard = 100	Total rel. DM yield	Digestibility	Rust susceptibility sept. 1989 1-5 ; 5 = no rust Lemtal = 3.3	Survival in artificial frosttests (rel. to Vigor)
Perennial ryegrass	1 intermediate	7	1	Merbo	107	98		97 NS
			2	½ (Amigo+Tresor)	104	104		
	2 late	6	1	Vigor	115	96		112 *
			2	Vigor	114	103		
Italian ryegrass	3	7	1	Lemtal	110	97	4.5	
			2		106	100	–	
	4	4	1		109	93	4.8	
			2		103	99	–	
	5	6	1		102	94	2.8	
			2		–	–	–	
	6	5	1		106	94	3.5	
			2		–	–	–	
	7 tetraploid	55	1		104	103		
			2		109	102		

TWENTY YEARS RYEGRASS BREEDING APPLICATING THE STANDARD BREEDING SCHEME

D. Reheul

Rijksstation voor Plantenveredeling, Burg. Van Gansberghelaan 109, 9820 Merelbeke

Introduction and methods

This as an assesment of more than 20 years breeding work with perennial ryegrass, executed according to the original strategy :

1) establish a polycross with selected individuals
2) test the progenies
3) construct a synthetic, recombining the genotypes with the best general combining ability (gca)
4) test the end product.

Some deviations on this scheme were practiced. The performance of a series of synthetics and their components is presented. Horizontal beams indicate DM yield, relative to a common standard.
As for the synthetics, the beams on the same height reflect data from a common trial.

These data and the current status of all the synthetics permit to assess the value of the breeding system.

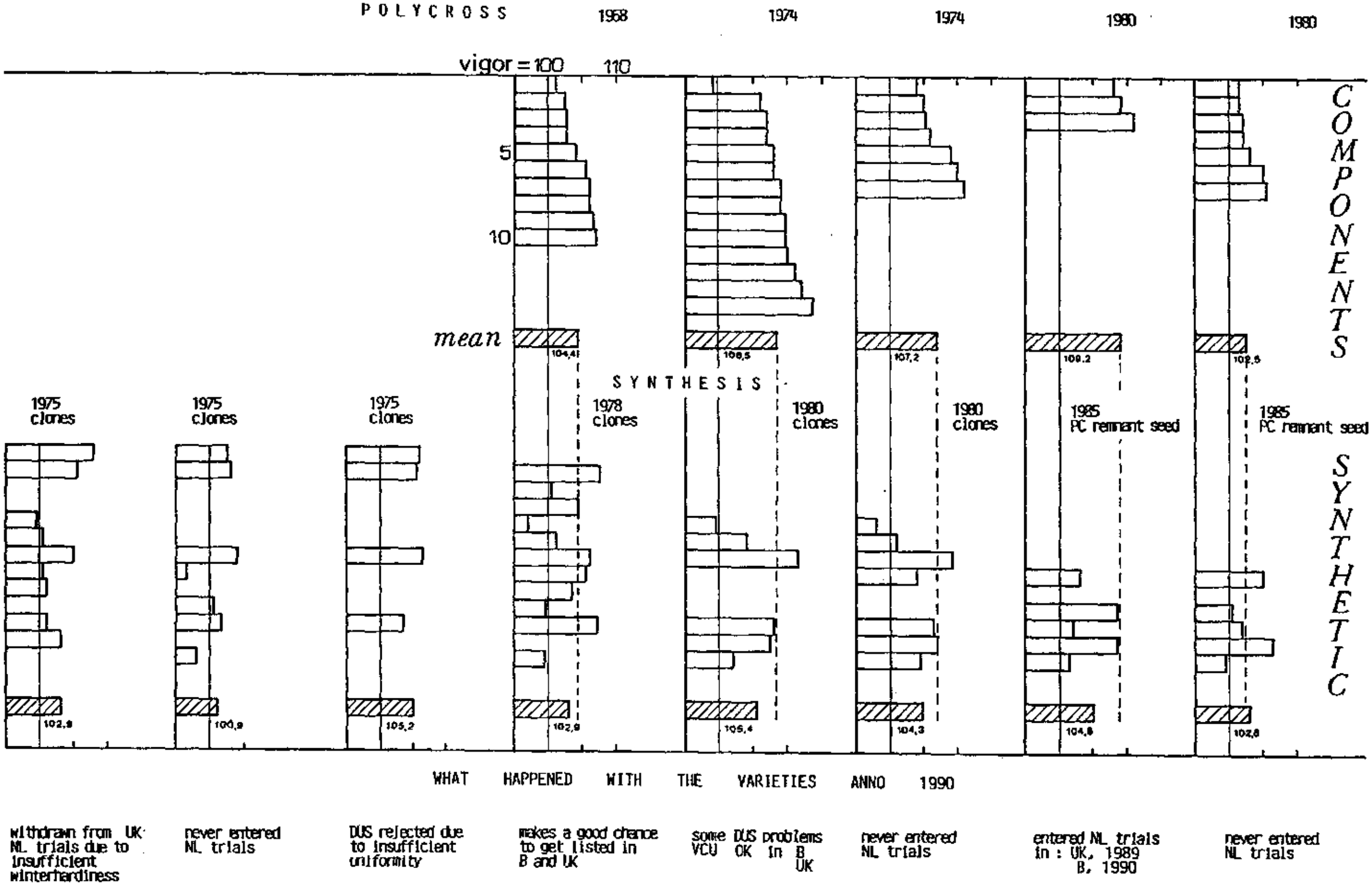
Results

See next page.

Conclusions

1. on average synthetics performed less than the mean of their components
2. The original system undoubtedly guaranteed a progress in DM yield independently of the number of components. It is time consuming.
3. Individual performance of the synthetics varied quite a lot. Averaging all individual performances narrowed differences between synthetics to maximum 5 units.
4. Omitting the gca test did not exclude the construction of a high yielding synthetic. Neither did the synthesis based on PC remnant seed instead of the original clones.

DM YIELD OF SYNTHETICS AND THEIR COMPONENTS



COMPARAISON BETWEEN BELGIAN PERENNIAL RYEGRASS ECOTYPES AND VARIETIES

Dirk Reheul

Rijksstation voor Plantenveredeling, Burg. Van Gansberghelaan 109, 9820 Merelbeke, België

Summary

A series of ecotypes collected in old grassland was tested for yield performance, digestibility and persistence. Most ecotypes were inferior to the varieties but some origins have valuable characteristics.

Introduction and methods

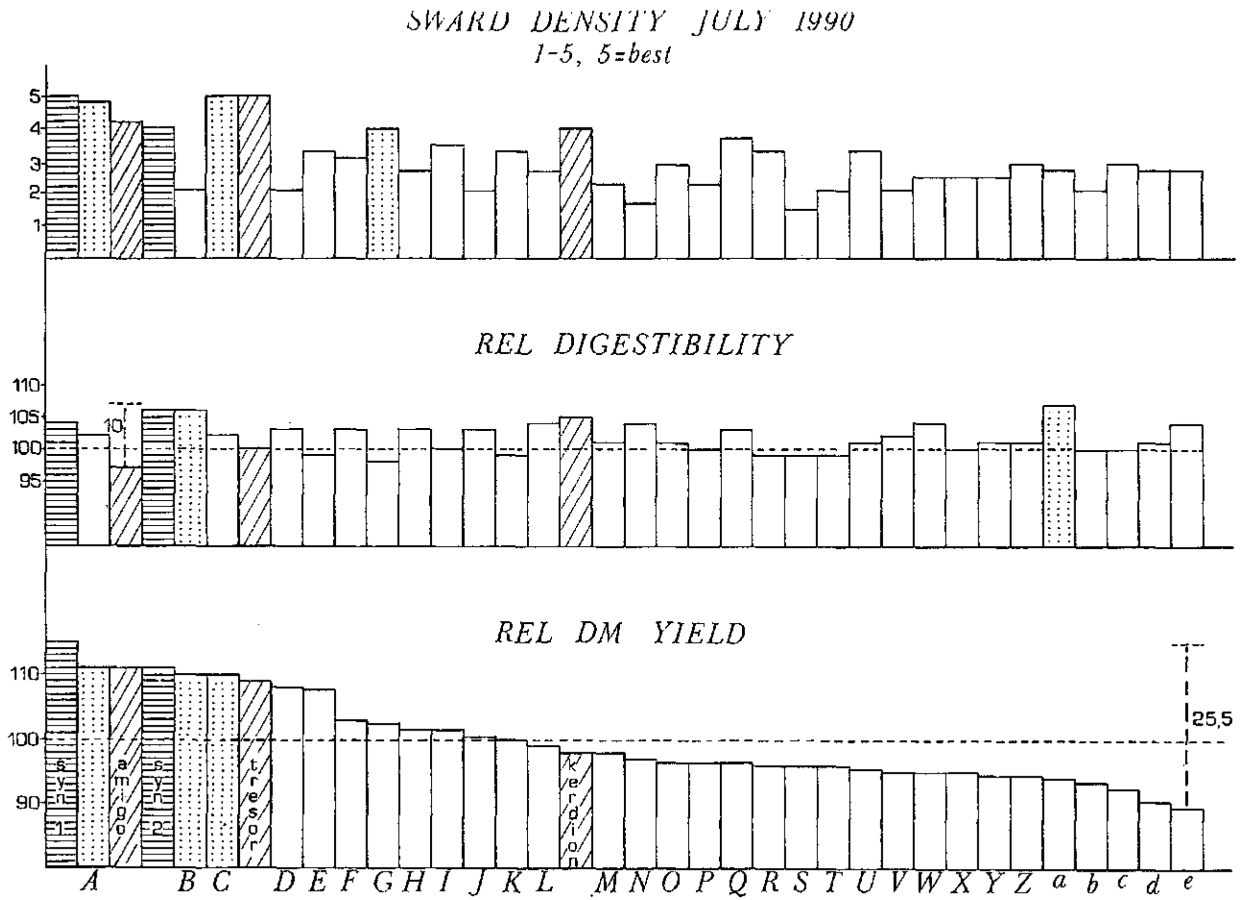
Natural grassland and old pastures in the coastal province were surveyed in 1986 and 1987. 31 pastures were sampled. The majority 'always had been grassland' but some pastures had been sown more than 30 years ago.
About 100 core samples were taken in March/April, replanted in isolated plots at RvP and allowed to set seed. In the spring of 1988 a trial with these 31 ecotypes and 5 varieties was installed. Over 1988, 1989 and 1990 10 cuts were taken. The digestibility of 3 cuts in the sowing year was analysed. Regrowth, drought tolerance during the dry summers of 1989 and 1990 and persistence were scored.

Results

See figure on p. 2.

Discussion

1. Apart from a few exceptions (A, C), the persistence was unacceptable low. The sward density suffered severely after the first spring cut and weeds (wich were rogued) introgressed.
2. Despite this low sward density, the yield performance of the better ecotypes didn't decline.
3. The variation in digestibility was small. Ecotypes B and a look promising.
4. If it holds true that old pastures are well adapted to grazing, the selection of surviving plants out of this mowing trial should generate a useful breeding population.


SWARD DENSITY JULY 1990
1-5, 5=best
5
4
3
2
1
REL DIGESTIBILITY
110
105
100
95
10
REL DM YIELD
110
100
90
25,5
syn 1
amigo
syn 2
tresor
kerdion
A B C D E F G H I J K L M N O P Q R S T U V W X Y Z a b c d e

SELECTION FOR SEED YIELD AND SEED YIELD STABILITY IN HIGH LATITUDE GRASS POPULATIONS

O.A. Rognli

Department of Biotechnological Sciences,
Genetics and Plant Breeding, N-1432 Ås-NLH, Norway

Summary

Divergent phenotypic selection for seed yield and seed yield stability gave large and highly asymmetrical responses. Cultivars responded very little to selection in high direction, while responses in local populations were larger than predicted, reflecting past selectional history of the populations. Higher phenotypic stability could be induced, especially by a combined selection of genotypes with high seed yield in the north (69°N) and high stability over locations.

Introduction

Grass cultivars from North Norway (above 65°N) are multiplied at lower latitudes, and are therefore vulnerable to genetic shifts during multiplication. Synthetic cultivars based on genotypes that show high phenotypic stability for seed production could reduce the risk of genetic shifts during multiplication (Rognli 1988).

Materials and methods

Seed yield was estimated in clonal experiments located at 60°N, 63°30'N and 69°N in Norway (Rognli 1988). Divergent phenotypic selections were established in timothy population 'Lakselv' (70°N) and cv.'Engmo' (69°N), in red fescue cv. 'Leik' (61°N) and the local population 'Alta' (70°N). Divergent selection for phenotypic stability was based on the GxL-interaction component, estimated by the ecovalence approach.

Results and discussion

Table 1. Response (%) to divergent selection for seed yield.

Entry	Seed yield	h^2	high % pred.	high % obs.	low % pred.	low % obs
Timothy:						
Lakselv	53.3	0.66	24.0	28.6	24.0	2.0
cv.Engmo	45.1	0.58	21.0	2.0	21.0	10.9
Red fescue:						
Alta	31.9	0.57	25.0	33.0	25.0	0.0
cv.Leik	46.3	0.72	24.0	10.6	24.0	15.5

Table 1. shows that while the observed response to selection for higher seed production has been larger than predicted in the local populations of both species, the selection response was much smaller than predicted in the cultivars. The pattern was quite opposite for the low selection lines. The results indicate that natural selection has selected for high seed production during several generations of multiplication of these old cultivars.

Table 2. Phenotypic stability of timothy and red fescue cultivars, populations, and high and low stability lines.

Entry	Seed yield g/plot	Stability parameters		
		W_i[1)]	b_i[2)]	s^2_{di}[3)]
Timothy:				
Lakselv	78.7	4.3	0.95	82.0
Lakselv high	88.0	2.7	1.06	48.5
Lakselv low	93.2	4.2	0.96	82.9
cv. Engmo	83.6	4.0	1.09	60.4
Engmo high	93.2	6.0	0.98	124.3
Engmo low	76.8	6.7	0.91	114.3
High stability[4)] + high seed yield in north	77.0	0.0	1.00*	1.2
Red fescue:				
Alta	71.2	10.8	0.62	247.5
Alta high	75.1	8.8	0.61	40.0
Alta low	91.8	10.6	0.69	452.4
cv. Leik	105.6	1.9	0.82	8.9
Leik high	106.2	0.0	1.02*	8.6
Leik low	125.1	12.0	1.41	227.6
High stability + high seed yield in north	102.9	2.2	1.19	13.1

[1)]ecovalence [2)]coefissient of regression [3)]residual sum of squares [4)]combined stability and yield selection (yield at 69°N) within 4 populations/cultivars.

Both higher and lower phenotypic stability could be induced, and responses were largest in red fescue, probably reflecting the more complex photoperiodic flower induction requirements of this species. Low stability lines are, except from 'Engmo', the highest yielding ones, confirming earlier results (Rognli 1988). The most stable populations resulted from a combined selection of genotypes with high seed yield in the north (69°N), and high stability over locations. This results have implications for the breeding strategy for higher latitudes.

References

Rognli, O.A. 1988. Estimation of genetic variation and stability of seed production characters by means of clonal experiments with natural grass populations. Proceedings of Eucarpia Fodder Crops Section Meeting, Lusignan, 1987, p.71-78.

CHARACTERIZATION FOR ESSENTIAL OIL AND MAIN CHEMICAL COMPONENTS OF TALL FESCUE VARIETIES UNDER STRESS CONDITIONS

M. Romani, A. Tava, N. Berardo, M. Odoardi, E. Piano

Istituto Sperimentale per le Colture Foraggere, Lodi, Italy

Summary

Three varieties of contrasting origin were submitted to stress conditions (drought and Nitrogen excess) in order to evidentiate influences on forage quality and palatability. The main components of DM and essential oil were examined. Differences were detected which originated both from varieties and treatments. The results are discussed in terms of varietal characterization.
Keywords: Festuca arundinacea, water stress, Nitrogen excess, composition.

Introduction

Abiotic stresses affect plant growth and chemical components of the plant as well as secondary metabolites, influencing plant quality and palatability. In the case of a forage crop, negative effects on nutritive value of the herbage and voluntary intake by animals may occur (Scehovic, 1988). Common environmental stresses are seasonal water deficit and high temperature. Mineral fertilization, particularly Nitrogen on grasses, may interfere with these climatic limitations resulting in an additional stress factor. This research was aimed at assessing the effects of a short-term water deficit, an excess of Nitrogen application, and their interaction on primary and secondary metabolites for three tall fescue varieties of contrasting origin.

Materials and methods

Plants of 3 cvs of tall fescue (Magno, M. Kasba and Tanit), grown in plastic pots, were subjected to the following treatments: normal supply of water and Nitrogen fertilizer (W N); water withholding with normal N supply (-W N); N excess (300 Units/ha) with normal water supply (W+N); combination of both water withholding and N excess (-W+N). After 8 days, when soil moisture approached the permanent wilting point, plants were cut and processed for chemical analyses. Dry matter percentage (%DM) and dry matter yield per plant (Y/p) were determined. Crude protein (CP), reducing sugars (RS), total soluble carbohydrates (TSC), acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber (NDF) and ash were also determined on DM basis. The green matter was analyzed for the essential oil components to characterize cultivar's response to the applied stress, as varietal differences have been found for several volatile components in tall fescue (Odoardi et al., 1989).

Results and discussion

Results on yield parameters and chemical components for each treatment are summarized in table 1, where (W N) represents the control not stressed. Table 1 also reports the significance of the mean effects for W and N factors and their interaction. Yield per plant and %DM were drastically affected by single treatments as water shortage alone (-W N) reduced total biomass, and N excess alone (W+N) increased it in respect to the control. An inverse effect was found for % DM. For both characteristics there was a significant interaction "N x W". Among chemical components, only TSC was significantly affected by water withholding, while N excess significantly modified all parameters, increasing CP, and reducing the others. Significant interaction between W and N treatments were found for RS, TSC and ADL.

Table 1. Mean values for each treatment of the parameters examined (cvs cumulated) and statistical significance of the mean effects of water (W), Nitrogen (N) and their interaction (W x N).

Parameters	Treatments				Significance of the effects§§		
	W N	W+N	-W N	-W+N	W	N	WxN
Y/p	30.27b§	40.93c	25.33a	28.58ab	**	**	**
%DM	29.14b	24.54a	33.21c	31.86bc	**	**	*
CP	9.13b	14.75a	8.03b	14.07a	ns	**	ns
RS	1.80a	1.01b	1.65ab	1.79a	*	*	**
TSC	7.20a	4.07c	5.89b	4.72c	ns	**	**
ADF	34.91a	32.89b	35.48a	33.13b	ns	**	ns
ADL	2.53a	2.03b	2.52a	2.49ab	*	**	*
NDF	61.44a	59.05b	61.42a	58.23b	ns	**	ns
Ash	9.54ab	9.13b	10.42ab	10.63a	**	ns	ns

§. Means within line followed by the same letter are not significantly different at P .05.
§§. Mean effects significance: (*)=P.05; (**)=P.01; (ns)= not significant

Besides Y/p, significant differences among varieties were found for the mean content of TSC, ADF and NDF. In addition there was a significant interaction "W treatment x variety" for RS, %DM and Y/p and "N treatment x variety" for RS and TSC. Concerning essential oil components, those which mostly differentiated the varieties were hexenylacetate, hexenol, phyton, phytol and p-vinylguaiacol. N excess affected particularly epoxy-ß-ionone and dihydroactinidiolide, while water deficit influenced hexenylacetate. There was an appreciable interaction "W treatment x variety" for hexenylacetate and phytol, and "N treatment x variety" for dihydroactinidiolide.

In conclusion, treatments influenced the production and accumulation of primary and secondary metabolites, affecting the main quality parameters responsible for the feeding value of forages. Evidence was acquired that varieties may differ for these metabolites and react differently to stress conditions. Further investigation is in progress on the matter.

References

Odoardi, M., N. Berardo & A. Tava, 1989. Influenza di stress termici e siccità sulla qualità di una specie foraggera. Atti Convegno S.I.G.A., Alghero. p. 79.

Scehovic, J., 1988. L'influence des stress sur la qualité des fourrages. Revue suisse Agric. 20(4): 205-210.

LUCERNE MUTATIONS USEABLE FOR INCREASING SEED YIELDS

Z. Staszewski, J. Jagodziński, B. Jakubowska, R. Osiński

Plant Breeding and Acclimatization Institute, Radzików, Poland[1]

Summary

New mutations were selected and their morphology and inheritance patterns were studied as well. Top flowering - tf, uncurved pistil - up and reduced keel petal - rk were transmitted by recessive single genes. Gene tf seems suitable for lucerne improvement because it causes stem termination which is positively correlated with seed yield per plant.
Keywords: lucerne, spontaneous mutation, stem, flower, inheritance, terminal growth.

Introduction

Seed yield depends on a variety's potential and on many nongenetic factors, so that a direct selection for seed yield resulted in little progress. There are not many varieties producing higher seed yields than Altfranken or Flamande lucerne, as reported in FNAMS Bulletin, 1988. While the plant habits of several crops (wheat, peas, horse bean, lupine) have been subjected to changes, the morphology of lucerne has remained very similar in past decades. To obtain progress in lucerne seed yield potential, it seems necessary to change the plant and flower morphology. Current information of an unfinished study of flower and stem modifications is presented here.

Results and discussion

Spontaneous mutations were selected and their inheritance was studied in the field experiments. The morphology of mutants follows. Top flowering tf - mutation causes flower buds to grow on the stem tops, thus leading to the termination of the stem growth. Usually, several raceme petals can grow from a single node on main stems. Uncurved pistil up: the pistil is straight during flower tripping and no flower explosion occurs. Reduced keel petal rk - mutation shows keel petals that are about 15 per cent shorter in comparison to the standard flowers. Also the pistils are usually about 10 per cent longer than the keel petals and this causes the flowers to open.

The segregation ratios given in Table 1 evidence that new mutations were transmitted as monogenic recessive characters. The traits depending on up and rk genes seem to be involved in the open corolla phenotype described by Barnes and Hanson, 1967. These traits can facilitate pollination by honey bees and improve seed production after the breeding method exploiting high level of heterosis is applied.

1. Present address: Department of Genetics, Plant Breeding and Acclimatization Institute, Radzików, 05-870 Błonie, Poland

Table 1. Segregation patterns for the tf, up and rk genes.

Phenotypes of crossed plants and crossing variant	The segregation number of plants		Probability for ℵ - square	
	standard	mutant	ratio	P()
Standard x tf = F_1	90	0	1:0	1.0
Plants tf selfed = F_2	157	84	2:1	0.5-0.7
Plants tf x Plants tf = BC_1	162	29	5:1	0.5-0.7
Standard x Plants up = F_1	614	1	1:0	0.95-0.98
(Standard x up) selfed = F_2	361	12	35:1	0.5-0.7
Standard x Plants rk = F_1	268	0	1:0	1.0
(Standard x rk) selfed = F_2	287	6	35:1	0.3-0.5
(Standard x rk) x rk = BC_1	77	12	5:1	0.3-0.5

Table 2. Characteristics of various phenotypes in 897 plants of cv. Radius space planted.

Phenotype	Plant share (%)	Seed weight per 100 racemes (g)	Seed weight per plant (g)	Seed number per pod	Plant height (mm)	Second growth (%)	Dry matter per plant (g)
Type tf	21.0	3.16	18.50	2.0	855	12.1	348.0
Intermediate	31.5	2.42	10.73	1.7	791	15.4	244.0
Non-tf	47.5	1.90	6.05	1.6	888	23.0	291.0
LSD (5%)		0.48	6.29	NS	NS	10.6	67.0

The results of table 2 show that plants of tf - phenotype are the best seed setters possesing good dry matter weight. This phenotype is characterized also by better uniformity and shorter period of flowering, so it can be helpful in breeding a population with more even ripening. This phenotype produces smaller second growth when the stems of the first growth are ripening. This is important in the case of a rainy summer. In addition, tf - phenotypes seem to have a distribution of plant food reserves favorable for pod and seed growth. It is almost certain that the plant habit of lucerne can be changed with the tf gene using a back-cross method. To breed a generative type population could become a realistic idea.

References

Barnes, D.K., Hanson, C.H., 1967. An illustrated summary of genetic traits in tetraploid and diploid alfalfa. U.S. Department of Agriculture Technical Bulletin 1370, Washington.

Bulletin FNAMS No. 104, 1988. La productivité grainière des variétés de Luzerne.

LIGHT AND TEMPERATURE RESPONSES OF PHOTOSYNTHESIS IN FOUR RYEGRASS CLONES DIFFERING IN PRODUCTIVITY

Ries de Visser and Riet de Kock

Centre for Agrobiological Research (CABO), Wageningen, The Netherlands

Summary

Cloned plants of four cultivars of Lolium perenne L. differing in productivity and nitrogen use efficiency (NUE) were studied. We examined whether photosynthesis contributes to varietal differences in growth rate. CO_2 exchange rate (CER) and chlorophyll fluorescence were measured. Under optimal conditions, no differences in CER were found. However, the CER of low-light (0.14 mmol/m^2.s) adapted plants of high yielding clones was less sensitive to conditions inducing photoinhibition (1.4 mmol/m^2.s and/or a 7 K temperature drop). We conclude that photoinhibition and CER contribute to variation in growth rate between ryegrass genotypes.
Keywords: clones, photosynthesis, photoinhibition, ryegrass, yield.

Introduction

Productivity and nitrogen use efficiency (NUE, g biomass produced per g N taken up; Chapin 1980) are positively correlated in L. perenne (Baan Hofman 1988). Comparing plant species, specific leaf area (SLA) and leaf weight ratio (LWR) are correlated with relative growth rate (RGR) rather than net assimilation rate (NAR; Poorter 1989). However, differences in morphology are smaller *within a species*. Furthermore, simulation studies show that a small increase in net photosynthesis dramatically affects dry matter accumulation. Also, potential photosynthesis rates as measured under optimal, constant conditions may be realized only rarely and to a variable extent by different genotypes in a dynamic, sub/supra-optimal environment. Here we examine the response of photosynthesis to the important fluctuating factors light and temperature, in clones of four ryegrass cultivars differing in productivity.

Material and methods

Cloned plants of Lolium perenne L. (line Selectie I, cvs. Parcour, Pelo and Splendor; relative yields 1.32, 1.26, 1.10, 1.0, resp.) were grown in aerated nutrient solution in a climated glasshouse at 18-20°C. CER was measured by IRGA in an open system. Maximal (F_M) and variable (F_V) fluorescence were measured at 293 K (cf. Somersalo & Krause 1988).

Results and discussion

Under optimal growth conditions, net photosynthetic rates were similar, with an 8% higher rate in cv Parcour than in cv Splendor (Fig. 1A). But this difference increased significantly (to 44%) after a (normally occurring) exposure to high light (10x) and a 7 K temperature drop (Fig. 1B). Chlorophyll fluorescence measurements and CER indicate significant differences in photoinhibition between high and low yielding cvs at 13°C (Fig. 2). Respiration rate was increased by a factor 2 during several h after the exposure to high light in all genotypes (results not shown).

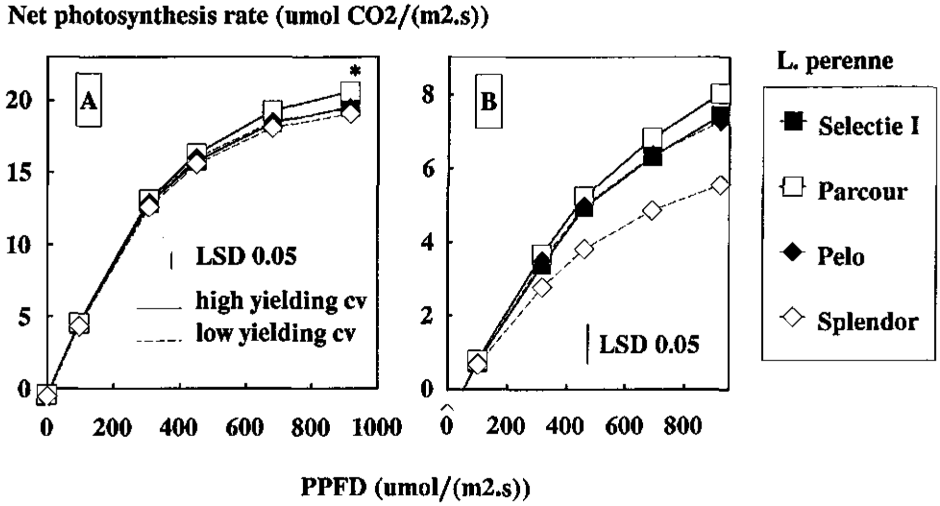
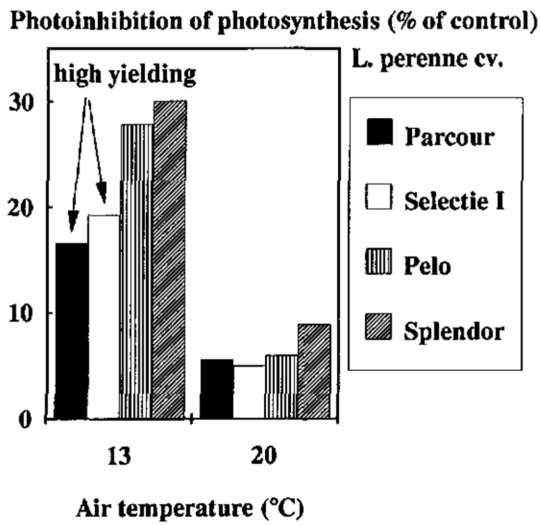


Figure 1. Light response of photosynthesis of ryegrass clones grown under near-optimal conditions (A) and of low-light (0.14 mmol/m^2.s) acclimated plants after a 2.5 h exposure to high PPFD (1.4 mmol/m^2.s; B).

Figure 2. Temperature effect on photoinhibition of net photosynthesis (cf. Fig. 1B) after a 2.5 h exposure to high PPFD (1.4 mmol/m^2.s).



These results indicate that photosynthesis contributes to varietal differences in growth rate, especially because the response of photosynthesis to (fluctuating) environmental conditions differs between genotypes. Screening of genotypes for high photosynthesis under various (adverse) conditions can be done rapidly by measurement of chlorophyll fluorescence.

Acknowledgements: We thank Dr JLP van Oorschot and Mr PH van Leeuwen for help with the fluorescence and photosynthesis measurements, respectively.

References:

Baan Hofman T 1988. CABO Report 1986, pp 31.

Chapin FS 1980. Annual Review of Ecology and Systematics 11: 233-260.

Poorter H 1989. *In*: Lambers H *et al.* eds, Causes and consequences of variation in growth rate and productivity of higher plants. SPB Acad Publ, The Hague, pp 45-68.

Somersalo S & GH Krause (1988) *In*: Lichtenthaler HK ed., Applications of chlorophyll fluorescence. Kluwer Acad Publ, Dordrecht, pp 157-164.

RECENT YIELD IMPROVEMENTS THROUGH RECURRENT HALF-SIB FAMILY SELECTION IN DIPLOID PERENNIAL RYEGRASS

P.W. Wilkins

AFRC-IGER, Welsh Plant Breeding Station, Aberystwyth, UK

Introduction

The usual method of improving dry matter yield in segregating perennial ryegrass populations is several generations of phenotypic selection, followed by a polycross or topcross progeny test for plot dry matter yield and persistency to select the several mother plants which constitute a new synthetic variety. In such a system, direct selection for plot performance is weak and is applied only once every ten years or so. There is little evidence to show that phenotypic selection is effective in combining increased yield with good persistency under plot conditions and thus there is a need for an alternative breeding system. Recurrent half-sib family selection could perhaps provide this since it enables a generation of direct selection for plot performance every three years. The present paper reports progress in performance under small plot conditions through recurrent half-sib family selection and compares the performance of the families with that of the second generation of a synthetic variety bred from the same gene pool by the standard method.

Materials and Methods

Ba10761 is a synthetic variety developed from heterotic hybrids by three generations of phenotypic selection followed by a polycross progeny test to select the four mother plants (Wilkins, 1986). In the autumn of 1985, 240 plants from the half-sib (polycross) family which showed the best combination of dry matter yield and persistency in the above progeny test were clonally replicated and grown in 200 mm pots outdoors over the winter. There were four plants of each clone. After culling clones which were particuarly susceptible to *Rhynchosporium* and *Erysiphe graminis* or segregating for ear emergence date, 130 clones remained. These were isolated in four pollen-proof glasshouse compartments and allowed to interpollinate at random, one plant of each clone per compartment. Some of the clones were completely sterile and only 57 yielded sufficient seed for subsequent field evaluation. In the autumn of 1988, the process was repeated except that only three plants of each clone were grown and that the plants were from the two best half-sib families from the previous generation. Of 240 clones, 170 were isolated and only 61 yielded sufficient seed.

Families and control cultivars were sown at 2.5 g of seed per 1 x 1 m plot (25 kg/ha) in randomised block trials, there being four replicate blocks in the first trial and three in the second. Plots were harvested and dry matter yields determined six times annually: during early April, at an estimated 67 D, and thereafter at 4-week intervals. Compound fertiliser (20:10:10 N:P:K) was applied as split dressings in March and after each harvest to give an annual total of 400 kg of N/ha. Percentage ground covered by ryegrass was determined visually 4-7 days after the final cut.

Results

In order to achieve high total annual dry matter yields under the management used it is necessary to combine good early spring growth, good reproductive growth and good vegetative growth. In the second generation of selection, the mean yield of all the half-sib families tended to be higher than those of Ba10761 although not signficantly so (Table 1). Variation in yield among half-sib families increased in proportional terms from the first harvest year (18%) to the third (37%) but the broad-sense heritability of this variation

Table 1. Mean dry matter yield (t/ha) and ground cover scores of 1 x 1 m plots of half-sib family lines and control cultivars. Figures in ( ) are expressed as a percentage of cv. Talbot.

	Second generation of selection (57 families)					Third generation of selection (61 families)	
	total dry matter yield				% ground cover	total dry matter yield	% ground cover
Family/cultivar	1987	1988	1989	total	1989	1990	1990
$\bar{x}$ of all families	19.31 (107)	15.96 (112)	10.31 (110)	45.87 (107)	76.6	17.29 (110)	42.7
Highest family	20.75 (111)	17.34 (120)	12.38 (132)	50.10 (117)	82.5	18.96 (121)	53.3
Lowest family	17.34 (93)	14.40 (100)	8.91 (95)	41.27 (96)	70.0	15.62 (100)	33.3
Ba10761	19.53 (105)	15.17 (105)	9.33 (99)	44.94 (105)	70.0	15.19 (97)	36.7
Magella	17.31 (93)	14.15 (98)	10.46 (111)	42.45 (99)	80.0	16.72 (107)	46.7
Talbot	18.65 (100)	14.40 (100)	9.41 (100)	42.95 (100)	75.0	15.66 (100)	46.7
Broad-sense heritability (%)	29	28	17	23	42	41	35
SED	0.77	0.69	0.83	2.04	3.29	0.77	3.65
P	<0.001	<0.001	0.013	0.002	<0.001	<0.001	<0.001

declined with time. Although all families and varieties covered the ground reasonably well, they varied significantly in this respect. Only first harvest year results are available from the third generation of selection. On this occasion, the mean yield of all the half-sib families was significantly higher than that of Ba10761. Families again varied substantially both in yield and in the proportion of ground covered and estimates of broad-sense heritability were high. Comparison of these 1990 results with the first harvest year of the previous generation of selection suggests an upward trend in yield.

Discussion

Recurrent half-sib family selection resulted in an improvment in annual dry matter yield beyond that achieved by the standard method. The 21% variation in yield between half-sib families in the third generation of selection and its high broad-sense heritability suggest that a further generation of selection would result in further improvement. The material will become increasingly inbred as selection proceeds and therefore two or more selection lines run in parallel, which would enable restoration of heterozygosity by hybridization between the lines, might produce the best results. The main limitation to the numbers of families evaluated, and hence the rate of progress, has been the production of sufficient seed from a sufficient number of mother clones. Perennial ryegrasses in general have rather poor seed set due to a heavy genetic load (Marshall & Ludlam, 1989), and it is therefore not surprising that severe problems with poor seed set because of inbreeding were encountered in the present work. A compromise has to be struck between selection for high seed production, which is necessary to maintain high mean seed yields, and selection for agronomic performance.

References

Marshall, C. & D. Ludlam, 1989. The pattern of abortion of developing seeds in Lolium perenne. Annals of Botany 63, 19-27.

Wilkins, P.W., 1986. Breeding for dry matter yield in perennial ryegrass by wide hybridization and recurrent selection. Proc. Fodder Crops Sect. Eucarpia for 1985, 25-30.

THEME 1

WHAT HAS BEEN ACHIEVED IN FODDER CROPS BREEDING?

Workshops

ACHIEVEMENTS AND PROSPECTS IN FODDER CROPS BREEDING - A WORKSHOP REPORT

M. O. Humphreys

AFRC Institute of Grassland and Environmental Research, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed, SY23 3EB, UK

It was generally agreed that significant improvements had been achieved in breeding fodder crops. However, these would not be fully appreciated if only total dry matter production was considered. Testing authorities should be persuaded to place more weight in their recommendations on breeding improvements in traits such as the distribution of seasonal yield (for example, increased mid-summer production after heading in maritime N.W. Europe), persistency and winter-hardiness (particularly in northern latitudes), disease resistance and drought tolerance (particularly in South and East Europe) and quality with regard to animal nutrition. Consistency of yield was important but the precise breeding objectives necessary to achieve this depended on local climatic conditions.

Ecotypic variation was still considered to be of importance in fodder crops breeding and gene banks should be encouraged to characterise their material as much as possible in order to provide more information of relevance to breeding.

Although it was thought that the construction of synthetic varieties would continue to be of importance in fodder crops breeding, it seemed likely that simpler forms of variety construction would receive increasing attention in the future. These should make better use of heterosis and provide more direct routes for the introduction of new biotechnology. The heterotic potential of wide crosses within grass species and the value of hybrid or semi-hybrid varieties within and between grass species had been demonstrated already. Double cross varieties of tall fescue were available in France. Continuing discussions with testing authorities would be needed to determine possibilities for the registration of novel varieties such as semi-hybrids.

The workshop adopted an optimistic view that further significant progress could be made in fodder crops breeding which, at present, is still relatively undeveloped.

RESULTS OF THE WORKSHOP ON YIELD-PROGRESS IN GRASSES AND CLOVERS.

Arend Kleinhout
Dansk Planteforædling A/S
Højerupvej 31, 4660 Store-Heddinge

It was concluded that the yield gains obtained during the last 30 years were mainly due to improved persistency - achieved through better winterhardiness, drought resistance, etc.

It was felt that the breeding of multipurpose varieties - adapted to a wide range of environmental conditions - was the major factor for the relatively slow progress in yield performance during this period.

It was also felt that the DUS-requirements had not had a significant negative effect on yield development.

The discussion on breeding methods were not very conclusive.

Some participants felt that significant improvements could still be achieved by using spaced plants whereas others preferred selection under competition.

Only a few of the participants stressed the importance of increased homozygosity on the overall efficiency of selection in grasses.

A few participants felt that F1-hybrids in grasses - on the basis of incompatability - had great potential and should play an important role in the coming decade.

In the future individual grass and clover varieties should be assessed in mixed swards and expressed in terms of animal performance.

No decision was taken on the variety x location x year interaction - or rather the lack of it - in the assessment of the value, and in the subsequent description of the varieties.

However, the need for an increased international cooperation on this issue was stressed - particularly in view of the rapidly mounting costs of running yield trials, but the discussion on priorities in future research proved to be inconclusive.

ACHIEVEMENTS IN FODDER CROPS BREEDING

Workshop on theme 1: grasses
Chairman: C.W. Van Den Bogaert

1. Has progress been made in grass breeding over the past 25-40 years?

   It was generally felt that the reported progress in DM-yield (about 0.5 % per year) was small but steady. Although it is mostly said that progress in other crops, e.g. cereals, is faster, some participants did not consider this to be proven.
   In important characters other then DM-yield as such, progress has been bigger; these characters are persistency, resistance to certain diseases, winterhardiness; they are negatively correlated with each other and/or with herbage yield; this negative correlation partly explains the slow increase in DM-yield, as does the complex nature of grasses.

2. How can the rate of success be improved?

   Most participants believe faster progress can be made by breeding varieties for specific conditions and special countries or regions within a country. Such varieties would be genetically narrower, and this would at the same time cause less problems in complying with DUS requirements. Some participants do not believe genetically narrow varieties will lead to bigger improvements, but they agree that DUS-requirements will not tolerate broad varieties in the future.

   Varieties with a narrow adaptation will reduce rentability of breeding work as many varieties, each with a small potential market, will have to be maintained, produced and marketed.

   It is believed that better selection and evaluation methods can also improve the rate of success. Research on breeding methods is important, as is the development of better methods to select artificially for winterhardiness and resistance to diseases, to reliably screen seedlings for adult plant characteristics, to assess herbage and seed yield properly.

3. Is DM-yield still the right and most important breeding goal?

   Yield, as it is presented in variety list figures, is the result of 2 or 3 years trials, and that is not really the long term yield as a farmer will experience and wish.

Therefor long terms yield results are important, hence persistence is a major goal.
Modern varieties will have to yield well with a low energy input, and then diseases are more likely to occur.

Digestibility is becoming very important, but the breeder badly needs fast, reliable screening methods on plant basis, before he can efficiently breed varieties with improved digestibility. What is actually asked for are varieties with a higher digestible DM-yield, but up to now nobody can say how much dry matter could be sacrificed in favour of a better digestibility. So in the end varieties are still judged primarily by the DM-yield figure as it is presented.

4. Do we still need a variety list?

Most participants believe a recommended list is in the interest of farmers and of breeders. A compulsory national list is somewhat more questioned.
Disappearance of a national and recommended list will benefit the big multinational breeding companies, who are able to perform their own testing of varieties and spend money on publicity to market their varieties. It remains to be seen whether in the end the few remaining jumbo companies will safeguard the importance of persistence and reliability of the varieties, or eventually favour the economically most rewarding ones (e.g. good seed yielders).

WORKSHOP ON LEGUME FORAGE SPECIES

Chairmans: M. Falcinelli, Italy and C. Mousset Declas, France

About 35 researchers attended this workshop.

It was generally felt that fodder legume species can be improved and bred for several different traits: yield per se, seasonal yield, persistence, stress and/or pest resistance, different management systems, adaptability to consociate growth, protein content and high digestibility are just some examples.

Due to so many factors, it appears extremely difficult to review generically the achievements and successes that have been done.

Forage varieties should be tested with respect to the traits and conditions in which they were selected; all results and estimated gains will be relative to these restraints.

Often fodder crops breeding has not achieved great levels of success because the available ecotypes are already very good, hence gains of only 5-10% in forage productivity are achieved with traditional methods. Furthermore these gains are difficult to quantify because forage yield should be evaluated in terms of meat, milk or wool production.

Usually good progress has been made by selecting for biotic and abiotic stress resistance, but little improvements appear possible with polygenic characters such as forage productivity.

Emphasis has been placed on the importance of evaluating and selecting new varieties in consociate conditions, if the fodder species have to be grown in mixed swards.

The most promising methods to improve the rate of success in forage crops breeding seem to be the selection in dense stands instead of as spaced plants, the use of restricted recurrent phenotypic selection that improves the effectiveness of standard schemes, the constitution of hybrid varieties to exploit the potency of heterotic vigour and the use of modern biotechnological techniques to overcome the limits of traditional methods.

Particular attention should be reserved to the opportunity of exploitation of the non-conventional reproductive pathways, such as apomictic mechanisms or the production of unreduced male gametes. This field is generally considered to offer very good perspectives for the future improvement of fodder crop species.

THEME 2

NOVEL STRATEGIES AND BIOTECHNOLOGY

Main papers

THE ROLE OF SELFING IN THE SYNTHETIC AND HYBRID VARIETIES CONSTITUTION IN FORAGE CROPS

P. Rotili

Istituto Sperimentale per le colture Foraggere, 20075 Lodi, Italy

Summary

The following species have been studied: *Medicago sativa*, *Trifolium alexandrinum*, *Lolium perenne*, *Lolium italicum*, *Festuca arundinacea*, *Dactylis glomerata*. A wide variability in inbreeding depression both for forage and for seed production has been noticed. The absence of inbreeding depression has been found in many cases either in legume or in grass species. Models concerning synthetic and hybrid varieties in diploid and tetraploid forage plants are proposed to discussion.
Keywords: heterosis - variety model, inbreeding depression, mating system.

Introduction

At present forage cultivars are all synthetic varieties; hybrid varieties-F1 commercial seed-are not on the market because up to now mechanisms have not yiet been found for the pollination control, which assure a satisfying result from the economic point of view. Much work has been done to prepare male-sterile material but with poor results on seed production. Other solutions, as manipulation and exploitation of the reproductive systems ,are now under investigation (Kobabe, 1989; Hayward,1989). As an alternative solution, we suggest the utilization of selfing and competition in order to constitute performant hybrid varieties, both for seed and forage production. The aim of the present contribution is the exposition and discussion of the reasons of such choice.

Reproductive systems of the principal forage crops

The genetic organization of the plant populations is the result of interactions among genome organization, spatial and temporal distribution of individual plants, seed dispersion, germination, and a combination of selective and random events. Although most of researchers indicate that the mating systems can be considered in a trichotomic context, that means predominant cross pollination, predominant self-pollination or both mixed, these systems are frequently presented and interpreted in a dichotomic context, with on one side the allogamous species, and on the other side the autogamous species. (Crude and Lyon, 1989). Nevertheless, the relative importance of selfing and cross pollination varies as among as within species in many genus (Jain et al., 1989). Selection for intermediate rates of self fertility can take place in structured populations when the migration is the result of seed dispersion, but it cannot when it results from pollen dispersion (Holsinger, 1986). Almost all the important forage species are allogamous. Nevertheless, within the species there is a great variability as in natural as in artificial conditions. At the Institute of Lodi in isolation conditions a significant number of populations or cultivars of the different following species presents many self-fertile plants: *Medicago sativa*, *Lotus corniculatus*, *Trifolii alexandrinum*, *resupinatum*, *pratense*, *repens* and *hybridum*, *Festuca arundinacea*, *Lolium italicum*, *Lolium perenne*, *Dactylis glomerata*.

One can ask what is the degree of cross-fertility of such plants in natural conditions. We have to underline that, up to now, the investigations with markers, on the natural reproductive systems of the different species are still unfrequent. Such a gap should be closed if one wants to choose the model for the variety constitution on a sure basis.

Selfing effect on Forage yield

The study concerned grass and legume species. The selfed plants have been chosen in dense sward and the inbreeding depression has been measured in the same conditions. In all the species the variability of inbreeding depression is large.

In Medicago sativa, the cultivars under investigation are: Florida, Cantoni, Leonicena, Friulana, Sewa, Equipe. Only 5 S1 families out of 80 show a little inbreeding depression (-3% and -5%). In Dactylis glomerata 35 S1 families of cv . Dora have been studied. The 26% of S1 families gives a forage yield wich is not different from the corresponding So. 36 S1 families were derived from Lolium italicum populations collected in permanent meadows ("prati stabili") more than 80 years old of the irrigated Lombardy. The 47% of these families gave the same forage yield as their corresponding So families. In Lolium perenne 7 S1 families out of 15 gave the same forage yield as their corresponding So. Such material had the same geographical origin as Lolium italicum. 28 populations of Trifolium alexandrinum (22 Italian and 6 Egyptian) have been studied. The 18% of S1 families gave the same production as the corresponding So.

Jain (1976) resuming the theoretical consequences of inbreeding in relation with the factors of evolution, underlines that its primordial result is a decrease of heterozygosis, with a decrease of the genetic load due to the masked recessives. Moreover, the drop of the recombination rate involves more important roles of the linkages and genic interactions, bringing to multiloci associations.

To explain the absence of inbreeding depression (or "tolerance for inbreeding consequences") we have formulated the following hypothesis: the concerned individuals have an average degree of homozygosis very high for the whole of genes favourable to the vigour. It is interesting to observe that the most sensitive species with regard to selfing are the leguminous species. This suggests that the type of pollination, anemophile for the grasses and entomophyle for the leguminous species, plays an important role. Hamrick (1989) studied the structuration in time and space of the genetic variation: he showed that the distribution of this variation can be predicted with some precision if one has at disposal information on the pollination and the seed dispersion. The species presenting a high genetic mobility (efficacious flying pollinators, seed largely dispersed) have in general values of diversity lower than the species with anemophile pollination and low seed dispersion.

In the grasses of natural meadows, included in this second group, two kinds of proximity are present: the proximity of pollen and the proximity caused by the seed fall, both favouring inbreeding and a certain tolerance to its consequences. In artificial meadows, where commercial seed is produced, the pollen proximity is surely important while the proximity by seed fall, although evidently present, has a poor effect because of the limited persistence of the crop. In the leguminous crops, the proximity is less important, either at the pollinic level (because of the pollinators) or at the level of seed fall because of the different modalities of its dispersion by different animal species. As a consequence, we will have a genic mixing increasing heterozygosis. As for grasses, for the leguminous crops as well, a distinction has to be made, between natural and artificial meadow conditions. Both the proximity effects produce inbreeding; at the same time, the biological interference assures the domination of the most vigorous individuals;these latter are So vigorous because they have accumulated genes and gene combinations favourable to vigour. The fitness of the dominants is consequently hygher than the fitness of the dominated individuals. This is true as far as diploid and tetraploid grasses are concerned. In lucerne, because of less important proximity effects, the plants not undergoing inbreeding depression are less frequent. This means that in this species the frequence of trigenics and tetragenics is always very high. Nevertheless, the variability in inbreeding depression is very high on all the species, diploid or tetraploid. This can be explained with the variability of the average heterozygosis degree of parental plants as well as with the gene quality and their interactions in the same plants.

Main advantages of selfing

Selfing allows us: 1. To identify the plants which are tolerant towards selfing; with these plants we can build successfully hybrid and synthetic varieties. 2. To homogenize the material. This is very important (chiefly for the lucerne meadow) as far as productivity, yield stability and persistence are concerned. 3. To select for additivity and therefore to increase the general combining ability (G.C.A.) of the parental plants. 4. To increase the variability at different levels of inbreeding.

Synthetic and hybrid varieties

The main objectives of the different breeding methods applied to the allogamous species and sometimes to the autogamous as well, is the accumulation of vigour connected both with the heterozygosis and the quality of genes and gene combinations. With regard to the practical utilization of heterosis, it is possible to distinguish two types of varieties: 1. The hybrid varieties resulting from the controlled hybridization of clones, lines or families genetically different. Such a type of variety allows the maximum exploitation of the variability in heterosis. 2. The synthetic varieties,resulting from the uncontrolled hybridization of the parental constituents, does not allow the maximum exploitation of heterosis.

Variety models

Three variety models are discussed. The material we used includes clones, lines or families derived by selfing during a number of generations compatible with a good reproductive ability and yield capacity of individuals. The first model allows the building of a single cross. The lines derive from clones selfed and therefore bred in competitive conditions for G.C.A. Such a single cross can exploit at its best the S.C.A. if the rate of allogamy is at the maximum level. We have to remind indeed that the competition in favour of allogamy is continuous: at level of pollen, embryo, seedling, plant. (Perrot et al., 1982). In addition, a strong inbreeding involves some self-sterility and therefore facilitates the allogamy (Rotili,1976). The single cross variety can be suitable only for diploids because in the tetraploids this model never will exploit the maximum heterozygosis when we utilise inbred material. But also in diploids the single cross is valid for forage production but not for seed production because the high cost of seed. The second model concerns a double cross made with two single crosses after their multiplication.

This model also in tetraploids allows the obtention of an high level of heterozygosis and, at the same time, the exploitation of the S.C.A.between the single crosses. The model number 3 represent a classical synthetic variety. The advantages and the disadvantages of this model are well known. We want only to remember the difference between diploids and tetraploids concerning their evolution during the generations of multiplication. In tetraploids, a decrease or an increase of vigour will be with regard to the number of parents, their degree of inbreeding and the floral biology. These elements are necessary but not sufficient. Indeed,it could be necessary to use clones derived from different parental populations having a high genetic distance. In lucerne, studyng the evolution of vigour through the generations of multiplication of different experimental synthetics, we found that all synthetics increased in forage yield from Syn 1 to Syn 2. On the contrary, in Syn 3 some synthetics showed a decrease in yield when compared with their respective Syn 2. No effect of the number of constituents was observed on this trend (Rotili et al., 1985). The evolution from Syn 1 to Syn 2 is in agreement with the theory concerning the autotetraploids; the loss in Syn 3 can be explained with the assumption that in Syn 2 the maximum level of heterozygosity is reached for some synthetics. In our experiment parental clones were derived from the same parental populations.

Selfing applied to lucerne breeding

We are exploiting the possibilities to increase the biomass productivity by using selfing and competition in the building of synthetic and hybrid varieties adapted to an intensive exploitation (six-seven cuttings per year) for the Po Valley and for the irrigated plains of Central Italy. The research work on the role of selfing in the lucerne breeding has to answer to the following questions: 1. Is it useful or not to utilize a phase of selfing in the breeding program? 2. If it is,how many generations are to be used? 3. Which variety model is the most efficacious? 4. A same variety model is suitable or not for both forage yield and seed production?

Concerning the role of selfing our results (Rotili,1976) show that:1. selfing allows a homogeneity of morphophysiological traits, which is very important for the persistence of meadow structure. 2. The use of selfing permits us to take into account the tolerance to inbreeding in the choice of plants. 3. Both the mean and variability increased with the level of inbreeding in diallel crosses derived from partly inbred parents; in the same materials, the G.C.A. and S.C.A. variances increased until the S2 level and remained stable until the S4 level; G.C.A. was always much higher than S.C.A.. The same results we had in diallel crosses derived from single crosses. 4. Theoretically, the vigour of single crosses among autotetraploid parents should decrease as their degree of inbreeding increases. However in our experiments, where selection was practised, the mean of single crosses increased with inbreeding. This gain was transmitted to the Syn 2 generation of synthetics indicating that the selection, in interplant interference conditions, of vigorous plants within vigorous progenies during the selfing phase was successful.

How many generations of selfing? Some plants reacted positively to selection and other did not. In addition, after the second generation of selfing further selection became ineffective. The following answers seem to be valid: the plants, whose vigour was due not only to the high degree of heterozygosity but to favourable genes and gene combination as well, reacted positively to selection until S2 level. In the succeeding generations, further progress in accumulation of favourable genes appeared to stop. An explanation may be found by analyzing the fertility.Our data have shown 80% of 285 vigorous plants at the S3 level to be almost self-sterile. At the S2 level this percentage was only 38%. It is evident,therefore, that self-sterility drastically limits inbreeding and selection. In order to accumulate favourable genes and gene combinations after two generations of selfing, a high degree of heterozygosity would have to be reconstituted by crossing. After that, selection and selfing could be made again.

Concerning the variety model, we have studied synthetic varieties, single and double crosses built with the same parental clones (Rotili and Zannone, 1976).The best double crosses are significantly better than the best single crosses at level of inbreeding S2, S3, S4. This result underlines the importance of the degree of heterozygosity in the expression of hybrid vigour in autotetraploid plants. In those plants the highest level of heterozygosity is given by the trigenic and tetragenic structures. Therefore, the objective of the breeder is the choice of a variety model allowing to hit this target. In addition the best double crosses derive from the clones which in the previous experiment showed the highest values of G.C.A.. Thus, it seems possible to obtain a good prediction of the double cross performance on the basis of the clones value measured by polycross progeny test. This fact is very important in the practical breeding. The comparison between the best 4-clone synthetics and the double crosses derived from the same parental clones, shows the double crosses overcoming the synthetics. Both the best synthetic variety and the best double cross are found at the S2 level of selfing:here the gain of the double cross over synthetic is 11% in two years.

From these results thirteen years ago we drew the following conclusions: the double cross model based on partly inbred parents in uncontrolled pollination seems to be the best solution for forage yield, but if we consider the seed production, it is questionable whether the advantage in forage production of the double cross over the 4-clone synthetics would be sufficient to justify the increased cost involved in producing hybrid seed. From our results (Rotili and Zannone, 1977) it is well evident that the pod fertility decreases with the increasing level of selfing, not only in single crosses but in double crosses as well. The forage yield of double crosses, on the contrary, increases with the increasing level of selfing, as we have said before. In any case, we have to underline that pod fertility of the double cross is not lower than that of 4-clone synthetic. We have seen before that the advantage of double crosses on 4-clone Syn 2 was 11%. Now we know that the 4-clone synthetics derived from partly inbred parents are not stable through the generations of multiplication. In fact, we observe an inbreeding depression of 5-10% when passing from Syn 2 to Syn 3. As the commercial seed is Syn 4, we can say that the advantage of double cross on 4-clone synthetic is about 20%. Is it such an advantage sufficient to make double-cross variety a more advisable solution than synthetic variety? We think that an over forage production of 20-25% could justify the utilization of double cross variety instead of corresponding synthetic variety. But if we want to take into account also the seed production, we have to utilise a hybrid variety corresponding to a 6-way or 8-way-cross. It is important to notice that the ferility is connected to levels of heterozygosis much more than the forage yield. The same is true for the synthetic variety: in this case the number of parents could be 6-8 partly inbred clones.

Conclusions

The utilization of selfing is efficaceous for both synthetic and hybrid varieties constitution in diploid and tetraploid species. Parental populations , clones, selfed and cross families must be evaluated under conditions of competition. It is important to know the degree of cross-fertility in natural conditions in the concerned genetic material. The first objective is the choice of the individuals tolerant for inbreeding consequences. At the end of the phase of selfing it is necessary to test the genetic distance of the chosen parental clones.

Using partly inbred material in diploid species, the best solution for seed and forage production is the double cross-variety. In tetraploid species the double cross is the best variety model for forage production, but not for seed production. A 6-way or 8-way cross variety seems to represent the good solution for both seed and forage production.

References

Cruden R.W., Lyon D.L., 1989. Facultative xenogamy: Examination of a mixed maring system. In the evolutionary ecology of plants, J.H. Bock et Y.B. Linhart (éds), Westview Press, Boulder, 171-207.

Hamrick J.L., Loveless M.D., 1989. The genetic structure of tropical tree populations: associations with reproductive biology. In:The evolutionary ecology of plants, J.H. Bock et Y.B. Linhart (éds) Westview Press, Boulder, 129-146.

Hayward, M.D., M.J. Kearsey, X. Xu and J. Chave, 1989. Outcrossing amongst inbred lines of Lolium perenne. XII Eucarpia Congress, Gottingen, Germany.Vorträge Pflanzenzüchtg,15,10-10

Holsinger K.E., 1986. Dispersal and plant mating systems: the evolution of self-fertilization in subdivided populations. Evolution, 40 (2): 405-413.

Jain S., 1976. The evolution of inbreeding in plants. Ann. Rev. Ecol. Syst., 7: 469-495.

Jain S., Molina F., Martins P., 1989. Evolution of mixed selfing and random mating: new solutions for an old puzzle? II Congress, European Society of Evolutionary Biology, Roma.

Kobabe G., 1989. A chance for chance-hybrids in fodder grasses (Lolium multiflorum) ? XII Eucarpia Congress, Gottingen, Germany. Vorträge für Pflanzenzüchtg, 15, 10-14.

Perrot V., Dommée B., Jacquard P., 1982. Etude experimentale de la concurrence entre individus issue d'autofécondation et d'allofécondation chez le thym (Thymus vulgaris L.). Acta Oecologica, Oecol. Plant., 3 (17), 171-184.

Rotili P., 1976. Performance of diallel crosses and second generation synthetics of alfalfa derived from partly inbred parents. I. Forage yield. Crop Science, 16: 247-251.

Rotili P., 1988. The concept of vigour and its application in a breeding program of lucerne. Eucarpia, Fodder Crop Section, Szarvas, Hungary, 64-78.

Rotili P., Zannone L., 1976. Performance of diallel crosses among single crosses of alfalfa and comparison of double crosses and synthetics derived from the same parental clones. Eucarpia Fodder Crops Sect. Meeting, Roskilde, 55-65.

Rotili P. and L. Zannone, 1977. Quantitative analysis of fertility in lucerne at different levels of selfing. Ann. Amélior. Plantes 27 (3), 341-354.

Rotili P., G. Gnocchi, C. Scotti and L. Zannone, 1985. Performance of second and third generation synthetics of lucerne derived from partly inbred parentes: forage yield and fertility. Eucarpia Fodder Crop Section, Svalov, Sweden, 135-141.

MANIPULATION AND EXPLOITATION OF THE REPRODUCTIVE SYSTEM IN FORAGE BREEDING

M.D. Hayward, D. Thorogood and J.G. Jones

AFRC.Institute for Grassland and Environmental Research,
Welsh Plant Breeding Station, Aberystwyth, Wales, UK

Summary

An understanding of the reproductive biology of the forages is essential for the effective exploitation of genetic variation. The genetic control of the various mechanisms controlling self-fertility incompatibility are discussed together with the manner in which they may be manipulated for the production of recombinant inbred lines and F1 hybrid cultivars.
Keywords: Incompatibility, self-fertility, Lolium, F1's.

Introduction

The production of improved cultivars of the forages depends upon the selection and exploitation of the considerable genetic variation which is known to occur within the natural progenitors of the species of interest, in derived varieties, or in closely related species. Studies have shown that this variation can be directly selected with the method of variety construction being conditioned by the reproductive system of the species concerned. If the species is inbreeding pedigree selection and inbred lines would be the system of choice, if outbreeding, synthetic cultivars are the traditional method of variety synthesis. Some forages, notably the tropical grasses, are apomictic allowing individual superior genotypes to be used directly as new cultivars.

As breeding progresses it is apparent that traditional systems of variety production are likely to become less efficient in making best use of the differing forms of gene action controlling the characters of interest. If additivity predominates inbred lines should be used; if dominance and/or epistasis, F1 hybrids. It must be emphasised however that F1 hybrids are in general merely an intermediate step before the fixation of all desirable genes in a superior inbred line. The production of inbred and/or hybrid cultivars generally necessitates manipulation of the reproductive system and thus requires a knowledge of the underlying mechanisms and their genetic control.

Control of the breeding system

Inbreeding

The ability to produce seed by natural self fertilization is restricted to a limited number of temperate forage species such as Bromus sp. and some of the mediterranean Loliums and Trifoliums. Little is known of the actual mechanisms involved in promoting self-fertilization other than that it generally necessitates modification of the flowering processes, such as by cleistogamy, whereby pollen is shed within closed florets. Clearly to manipulate such systems for the production of F1 hybrids will require the promotion of open-flowering, and the exsertion of stigmas and anthers to encourage cross-fertilization together with some means of pollination control.

Outbreeding

The majority of forage species are outbreeding with a genetically controlled incompatibility system. In the legumes as typified by Trifolium repens a single S locus gametophytic system is operational whilst in some of the Brassicae it is sporophytic in action. In the forage grasses which have been examined in any detail a two locus SZ, gametophytic system has been identified; incompatibility, which is expressed on the stigma surface, occurs whenever the SZ alleles in the pollen are matched by those of the stigma (Lundqvist, 1961). A large number of alleles are to be found at each locus. In Lolium for example it is estimated that between 30 and 40 alleles occur at both loci (Fearon et al., 1983). A consequence of this is that in most systems of variety synthesis adequate allelic diversity is present to promote full cross-pollination. Unlike single locus systems the grass mechanism does not break down with increase in ploidy, identity of a single SZ pair of alleles being sufficient to lead to incompatibility (Fearon et al., 1984). A consequence of this is that when the numbers of alleles are restricted, such as occurs when a cultivar is based on the progeny of a single pair cross, the level of cross-compatibility is lower in a tetraploid than in the corresponding diploid cultivar. This has been shown to account for reduced seed production in some tetraploid hybrid Lolium perenne x L.multiflorum cultivars (Hayward, Stephens and Jones, unpublished results).

Manipulation of the breeding system

Incompatibility to self-compatibility

The potential utility of inbred lines has lead many breeders to investigate the possibilities of producing inbreds in a wide range of grasses and legumes; to do so however requires an effective means of overcoming the incompatibility mechanisms. The most extensive work is undoubtedly that which has been applied to Lolium sp. since Jenkin (1931) showed that self seed could be obtained in L.perenne by enforced self pollination. Since then numerous experiments have examined the production of inbreds and the effects of the inbreeding process on the fertility and agronomic potential of the resulting lines (eg. Utz and Oettler, 1976; Jones and Jenebzadeh, 1981). In general fertility was found to be genotype dependent and that highly self-fertile material could be obtained. It was also apparent that the strong selection which accompanied this selfing process, coupled with inbreeding depression per se, lead to a very restricted range of potentially useful variation being obtained. In order to ameliorate this problem it was proposed by Nitschze (1983) that self-fertility could be transferred to the outbreeding L.perenne and L.multiflorum from the inbreeding species L.temulentum by a backcross procedure. Whilst the techniques available at the time limited this approach it was able to show that such transfer was feasible. Using the more recently developed methods for the study of self-incompatibility, namely UV fluorescence techniques, we have been able to identify the mode of inheritance of self-fertility (Thorogood and Hayward, 1988). It is by a single gene which linkage studies have shown to be either allelic to or closely associated with the Z locus of the incompatibility system. It has now been successfully transferred into both L.perenne and L.multiflorum by backcrossing and is being used for the production of inbred lines.

As an alternative system to the use of L.temulentum we have recently examined the control of self-compatibility in some inbred lines of L.perenne which have been obtained by enforced selfing. Analysis of the segregating generations derived from the cross of a pair of inbreds identified a single major gene controlling self- compatibility (Thorogood and Hayward, 1990). This gene, Sc/1, is independent of the incompatibility loci and may well represent a relict gene of what was originally a multilocus system of incompatibility. One immediate advantage of this gene was the associated high levels of seed production that could be obtained when it was operative. As such it has been incorporated into alternative genetic backgrounds and is now being exploited in a practical breeding programme.

Production of specific incompatibility genotypes

In order to use the incompatibility system for the development of hybrid cultivars it is necessary to be able to produce specific incompatibility genotypes unless, as in the case of Phalaris, the species is one where vegetative propagation of parental clones can be carried out on sufficient scale to allow commercial quantities of seed to be obtained (McWilliam, 1974). The main systems for exploiting incompatibility require the production of genotypes which are homozygous for the alleles at both of the incompatibility loci and may be obtained by breaking down the incompatibility barriers by selfing, or by the use of anther culture techniques for the production of dihaploids.

Exploitation of the reproductive systems

The potential of recombinant inbreds

The most extensive studies of the utility of inbred lines are undoubtedly those concerned with Lolium perenne (Utz and Oettler, 1976; Devey et al., 1987). These studies have examined the performance of the first cycle of inbreds produced by selfing populations or cultivars. In general it has been found that the lines are of low vigour for both characters of agronomic and seed production importance. This has been attributable to severe inbreeding depression reflecting the genetic load that these species posses and the necessity to overcome incompatibility. Second cycle inbreds have recently been produced at the Welsh Plant Breeding Station by intercrossing of some pairs of lines carrying the self-fertility gene Sc/1. Analysis of the F3 generation has shown that in small plot trials some recombinant families perform as well as the control cultivars (Xu, 1989). Biometrical genetic analysis revealed the presence of genetic variation for yield and the potential for producing even better performing lines. By pedigree selection it is thus possible to develop high performance, uniform inbreeding cultivars.

The production of hybrid cultivars

Several procedures are available for the production of hybrid cultivars in both inbreeding and outbreeding species of forages. A prime requirement for any hybrid programme is some means of pollination control. For inbreeding species this generally necessitates some form of male sterility mechanism which may be genetic, cytoplasmic or even chemically induced. In outbreeders hybrid development has concentrated on the utilization of the incompatibility systems which can be manipulated to allow varying levels of hybridity to be attained ranging from 50% hybrids to full double or single cross hybrids.

The potential of 50% hybrids has been examined in some detail by Foster (1971) who proposed that by mixing together two cultivars of similar flowering time random pollination would lead to the production of a progeny consisting of 50% intercultivar crosses. Although it was not possible to show the exact proportions of hybrids produced yield trials indicated the presence of hybridity through the heterosis expressed.

In order to raise the level of hybridity above the 50% level England (1974) put forward a system which involved a single generation of selfing of a genotype heterozygous at both loci (Hom0) followed by random mating of the resultant progeny over a number of generations in order to multiply up seed stocks. If the I1 generation is in the expected Mendelian proportions the advanced generations will have only a 50% level of within population pollen cloud compatibility. Intermating of two such populations will give rise to an 83% level of hybridity. This approach has been found to give rise to very encouraging levels of heterosis for dry matter production in perennial ryegrass (Posselt, pers comm); success however will depend upon having parental lines which show good combining ability.

In tetraploid species where a two locus gametophytic SZ system of incompatibility is operative greater levels of hybridity can be obtained. By selection of Hom1 genotypes at the diploid level prior to making them tetraploid, it is possible to reduce the level of within population compatibility to the order of 6-10% ie. effectively sterile. Here again mixed populations should give rise to virtually complete hybrids (Hayward, 1988).

The production of complete hybrid cultivars at the diploid level using incompatibility can be achieved by simply isolating together superior clones and allowing them to interpollinate. For such a method to be commercially successful does however require some means of vegetatively propagating the parental clones as in *Phalaris* (McWilliam, 1974). Alternatively mass embryogenesis using single cell culture systems would appear to have considerable promise in this area. Where such means are not available we have to consider the possibilities of manipulating the existing incompatibility system to allow seed multiplication and hybrid production. A number of schemes have been put foreward involving the development of homozygous incompatibility genotypes (Hayward, 1988). In a recent series of experiments appropriate material has been produced by anther culture or by selfing. By isolating suitable combinations of genotypes both single and double cross hybrids have been produced with complete hybridity resulting in all cases. To be commercially successful these systems do require the production of large numbers of the parental genotypes. Whilst anther culture seems to offer the greatest prospects the genotype dependency of the process and the high levels of male sterility and reduced vigour encountered in androgenetic plants of *Lolium perenne* would currently limit the widespread adoption of this method. Hom2 genotypes produced by selfing individuals of known incompatibility genotype have proved more effective. These plants retain a high level of heterozygosity and are thus much more vigorous in their seed production capacity. In terms of F1 hybrid concept such plants will give rise to hybrids whose expression of gene action will depend upon the level of homozygosity and dominance relationships of the loci controlling the characters of interest.

True F1's can only be obtained by the hybridization of inbred lines and in this respect we have examined the potential of inbred lines of normally outbreeding perennial ryegrass to retain the capacity for preferential outcrossing when two lines are allowed to interpollinate. In a series of differing trials it has been found that some lines will give rise to complete hybrid progenies (Hayward et al., 1989; Xu, 1989). Even in those lines which do produce some inbred seed the extent to which such individuals would contribute to the sward is likely to be minimal because of the intense competition which occurs.

Conclusions

The self and cross fertility mechanisms examined here and the possible means of exploiting them is dependent on our current state of knowledge of these naturally occurring reproductive systems of the forages. As our understanding of these processes increase further novel methods will no doubt be developed. In this respect apomixis is a system which is receiving increasing attention and will undoubtedly have a major impact upon breeding methods. To exploit any of these technologies will require an integrated approach to all the phases of a breeding programme.

References

England, F.J.W. 1974. The use of incompatibility for the production of F1 hybrids in forage grasses. Heredity 32: 183-188

Fearon, C.H., M.D. Hayward & M.J. Lawrence, 1983. Self-incompatibility in ryegrass. V. Genetic control linkage and seed set in diploid Lolium multiflorum Lam. Heredity 50: 35-45.

Fearon, C.H., M.D. Hayward & M.J. Lawrence, 1984. Self-incompatibility in ryegrass. VIII. The mode of action of S and Z alleles in the pollen of autotetraploid Lolium perenne L. Heredity 53: 415-422.

Foster, C.A. 1971. Interpopulational and intervarietal hybridization in Lolium perenne breeding: heterosis under non-competitive conditions. J. agric. Sci. Camb. 76: 107-130.

Hayward, M.D. 1988. Exploitation of the incompatibility mechanism for the production of F1 hybrid forage grasses. Euphytica 39: 33-37.

Hayward, M.D., M.J. Kearsey, X. Xu & J. Chave, 1989. Outcrossing amongst inbred lines of Lolium perenne. Vortrage fur Pflanzenzuchtg 15: 10-10.

Jenkin, T.J. 1931. Self-fertility in perennial ryegrass (Lolium perenne L). In: Self and cross-fertility and flowering habits of certain herbage grasses and legumes. Welsh Plant Breeding Station Series H 12: 100-119.

Jones, R.N. & P. Jenabzadeh, 1981. Variation in self-fertility flowering time and inflorescence production in L.perenne L. J. agric. Sci., Camb. 96: 521-537.

Kearsey, M.J., M.D. Hayward, F.D. Devey, S. Arcioni, M.P. Eggleston & M.M. Eissa,

1987. Genetic analysis of production characters in Lolium. Triple test cross analysis of spaced plant performance. Theor. Appl. Genet. 75: 66-75.

Lundqvist. A. 1961. Self-incompatibility in Festuca pratensis Huds. Hereditas 47: 542-562.

Lundqvist. A. 1975. Complex self-incompatibility systems in angiosperms. Proc. Roy. Soc. Lond. B. 188: 235-245.

McWilliam, J.R. 1974. Interspecific hybridization in Phalaris. Proc. 12th Int. Grassld. Congr. III: 901-906.

Nitzsche, W. 1983. Inheritance of the mode of fertilization in Lolium spp. Z. Pflanzenzuchtg. 90: 243-248.

Thorogood, D. & M.D.Hayward, 1988. Transfer of self-fertility from Lolium temulentum to L.perenne and L.multiflorum. Heredity. 61: 288.

Thorogood, D. & M.D. Hayward, 1990. The genetic control of self-fertility in an inbred line of Lolium perenne L. Heredity (submitted).

Utz, H.F. & G. Oettler, 1978. Performance of inbred lines and their top-crosses in perennial ryegrass (Lolium perenne). Z. Pflanzenzuchtg. 980: 223-229.

Xu, X. 1989. The genetic assessment of inbred lines and their hybrids in Lolium perenne L. Ph.D. thesis. University of Wales.

THE DIPLOGAMETE METHOD AND PLOIDY MANIPULATION IN FODDER CROPS BREEDING

R. Lumaret

Centre Emberger CNRS, BP 5051, 34033 Montpellier cedex, France

Summary

Sexual polyploidization has been shown to be an efficient means to transfer heterozygosity from the diploid to the tetraploid level, more particularly if first division restitution (FDR) mechanisms are involved. Recently, screening for 2n gametes has increased in legumes and has been extended to several grass species. The recent use of enzyme markers and of flow cytometry makes this screening still more efficient and substantial numbers of 2n pollen and 2n egg producers have already been found in many species. The use of such gametes in either unilateral or bilateral sexual polyploidization constitutes a very promising breeding method for fodder legumes and grasses in the near future, insofar as genetically broad initial diploid material is available.
Keywords: polyploidization, diplogamete, genetic diversity, plant breeding, fodder crop.

Introduction

In fodder crops, increases in yield, persistence and disease resistance have often been obtained through the use of polyploid cultivars. Somatic chromosome doubling by colchicine, nitrous oxide treatments or protoplast fusion and unilateral (4x-2x and 2x-4x) or bilateral (2x-2x) sexual polyploidization with 2n gametes have been utilized to produce polyploids in several legume and grass species. The recent application of sexual polyploidisation to grasses and the improvement of techniques designed to detect 2n gamete prodution, i.e. the utilization of enzyme markers and flow cytometry, provide a basis for this comparative assessment of recent results obtained for fodder plants from in relation to several means of polyploidisation introduced above. In particular, the probability of transferring linkats and heterozygote combinations from the diploid to the tetraploid level will be considered.

Performance of polyploids obtained from somatic and sexual polyploidisation in fodder crops

Comparison of performance between somatic and sexual polyploids is effective only if these polyploids are derived from the same genetic background (i.e. the same diploids). Fresh and dry weight production by somatic and sexual polyploids derived from the same diploid genotypes have been studied in several legume species (Pfeiffer & Bingham, 1984; Taylor & Wiseman, 1985; McCoy & Rowe, 1986). The superiority of the sexual polyploids in relation to the somatic polyploids was clearly evident. This can be attributed to the fact that somatic doubling of the chromosomes in diploids produces tetraploids with a maximum of 2 alleles per locus, whereas heterosis in outbreeding autopolyploids (most of the important fodder crops belong to this category) is dependent on maximum heterozygosity (Bingham, 1980). Isozyme analyses have confirmed the presence of multiple alleles at a locus in several natural autopolyploids, e.g. Medicago sativa (Quiros, 1982) and Dactylis glomerata (Lumaret, 1984). Quantitative genetic theory (Demarly, 1963; Gallais & Guy, 1970) and experimental studies (Dunbier & Bingham, 1975;

Tomekpe & Lumaret, 1991) have demonstrated the importance of tri- and tetra-allelic loci in increasing yield in fodder crop. This suggests that non-additive gene effects may be an important component of the variation. At present, the use of 2n gametes, more particularly those formed by first division restitution (FDR), and somatic hybridization via protoplast fusion, are the two available methods for maximizing heterozygosity. Somatic hybrids have been reported in alfalfa (Teoule, 1983) but regeneration from protoplasts is usually limited to only a few genotypes (McCoy & Walker, 1984) which would have to be propagated vegetatively for commercial purpose.

The search for 2n gametes in fodder crops

Up to now, 2n gametes have been found in all fodder crops in which their production has been investigated. The occurrence of such 2n gametes can been ascertained directly e.g. the examination of pollen size undertaken in Medicago ssp. (Vorsa & Bingham, 1979; McCoy, 1982; Veronesi et al., 1988, 1990), in Lolium perenne (Den Nijs & Stephenson, 1988; Salat et al., 1989; Wagenvoort et al., 1990) and in Dactylis glomerata (Van Santen, 1988; Maceira, 1990), and the examination of both pollen size and shape as in Trifolium pratense for which haploid grains are oblong whereas 2n grains are tetrahedral (Taylor et al., 1976; Parrott et al., 1985; Parrott & Smith, 1984, 1986a). Screening for 2n pollen based on size can be a practical method provided that it maintains a close correlation with ploidy level. In legumes, 2n pollen is substantially larger than normal pollen, thus facilitating the screening process which can be carried out thanks to a mathematical model, as proposed by Veronesi et al. (1988) for alfalfa. In grasses, and more particularly in Dactylis glomerata L., sceening based on pollen size is more difficult because of a wider overlap of diploid and haploid pollen size distributions so that only individuals producing a substantial amount of 2n pollen (determined by another indirect method) are usually screened by using the proportion of pollen grains greater than a threshold size (Maceira, 1990). For Lolium perenne, Jansen & Den Nijs (1990) have proposed to use a statistical analysis to estimate 2n pollen frequency.

In fodder crops, numbering the 4x individuals in the progeny of reciprocal 2x-4x crosses has been the predominant method used to determine and quantify the occurrence of 2n gametes in the diploids. Such experimental processes have often been conducted following the scenario presented in figure 1.

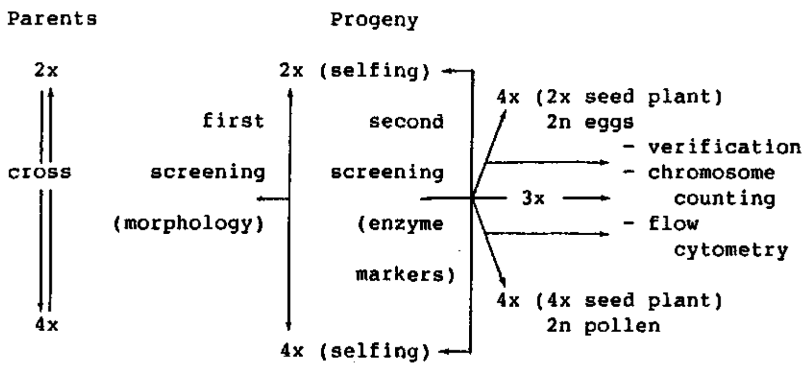


Fig. 1. Diagram representing the experimental protocol used to screen for 2n gamete producers from 2x-4x reciprocal crosses in fodder crops.

Since the study of 2n gamete production has mostly involved species that are not exclusively allogamous, to avoid selfing, or to aid identification of the progeny obtained from selfing or cross fertilization, either (a) 4x male-sterile plants have been used as female parents, e.g. in Medicago sativa (Bingham & McCoy, 1979; McCoy, 1982; McCoy & Rowe, 1986; Veronesi et al., 1986) and in Dactylis glomerata (Van Santen, 1988; Maceira, 1990) or (b) morphological traits with a recessive genetic determinism, such as "white flower" in red clover (Taylor & Wiseman, 1987), "cream colored flower" in alfalfa (Ffeiffer & Bingham, 1983) and lack of teeth along leaf margins in Dactylis glomerata (Maceira, 1990) have been utilized. Moreover, parental genotypes of known constitution at several enzyme loci can be chosen in 4x-2x crosses on the same purpose, e.g. in Dactylis glomerata (Lumaret et al., 1989; Maceira, 1990). In addition, observation of zymograms produced by electrophoresis provides a preliminary identification of the progeny ploidy level, e.g. in Dactylis (Maceira, 1990). Following this, progeny ploidy level can be verified by chromosome counts in root tip squashes or, such as in Dactylis, by nuclear DNA quantification from leaf tissue using the flow cytometry technique (Lumaret et al., 1989; Maceira, 1990) which is extremely efficient for screening among large numbers of plants.

In several fodder crops, 2n gametes have been screened for plants from wild diploid populations. In Medicago coerulea and M. falcata, 2n pollen frequency was found to range from 0.5 to 4%, and from 46 to 100% of the same plants produced 2n eggs with frequencies reaching up to 12 or 28% depending on the origin of the population (Veronesi et al., 1986). In the same material, a further screening based on pollen size revealed high frequency of pollen reaching up to 83% in M. falcata (Veronesi et al., 1988). In Dactylis glomerata the screening for male and female 2n gametes has been conducted for natural populations of nine distinct subspecies. From 20 to 75% of the individuals produced 2n-pollen, depending on the subspecies, with an average 2n pollen frequency of 3.02%. Six individuals produced exceptionnally high frequencies of 2n pollen, ranging from 8 to 14% (Maceira, 1990). Plants producing 2n eggs were detected in 47% of the diploids which produced progeny in 2x-4x crosses, with an average 2n egg frequency of 1.5%. Individual frequencies did not exceed 3.5%, with the exception of 26% in one plant. As also stressed by Veronesi et al. (1986) for alfalfa, diplogynous and diploandrous gamete production were not correlated with each other.

Moreover, substantial 2n gamete production has been found in breeding material of several fodder crop species. In Dactylis, 2n eggs were found in clones at frequencies of 17-100%, whilst five clones produced 2n pollen at a relative frequency of 3-98% (Van Santen et al., 1986). In Lolium perenne, out of 900 individuals (cultivars and inbred material), 20 plants produced 5-26% large (2n) pollen (Den Nijs & Stephenson, 1988). In alfalfa, a few clones were reported to produce more than 50% 2n pollen. In red clover, a synaptic mutant producing high frequency of both 2n pollen and 2n eggs has been identified and used successfully to produce 4x progeny in bilateral polyploidization (Parrott & Smith, 1984; Parrott et al., 1985). Several studies, meanly in alfalfa and ryegrass, have shown that environmental factors, such as temperature, can have significant effects on occurrence and frequency of 2n pollen for specific genotypes (McCoy, 1982; Den Nijs & Stephenson, 1988) but it remains difficult, in many cases, to associate a specific environmental effect with 2n pollen production.

Cytological base and genetic determinism of 2n gametes in fodder crops

The results obtained from several species of legumes and grasses are summarized in Table 1. Several meiotic mechanisms are involved which are genetically equivalent to either first division restitution (FDR) or second division restitution (SDR). In FDR gametes 100% of the heterozygous parental loci from the centromere to the first cross-over and one-half of heterogygote loci beyond the first cross-over are present. On average, a maximum of 40% of the parental heterozygosity is transmitted to the 2n gamete derived from SDR (Hermsen, 1984). As only preliminary cytogenetic research has been carried out in grasses, mostly in Lolium and Dactylis, it is likely that the several more mecanisms responsible for the formation of 2n gametes may not yet have been discovered.

Table 1. Origin of 2n gametes by either FDR or SDR and the occurrence of a triploid block in several fodder crops.

Species	2n gamete		Triploid block		Ref.
	pollen	egg	2x ♀	4x ♀	
Medicago sativa	FDR (Ps) JP	SDR (lc)	+++	+++	a,b,c
complex M. sativa coerulea falcata	FDR (Ps)	SDR (lc)	+++	+++	d,e
Trifolium pratense	FDR (sy)	-	+++	+	f,g
Lolium perenne	SDR, JP	-	-	++	
Dactylis glomerata	SDR (lc)	-	++	+	h,i,j k,l,m

Ps: parallel spindle; sy: synaptic; lc: lack of cytokinesis; JP: jumbo pollen; + + +: very strong; + +: strong; +: light; -: no data.
a: Vorsa & Bingham, 1979; b: McCoy, 1982; c: Pfeiffer & Bingham, 1983; d: Veronesi et al., 1986; e: Tavoletti et al., 1989; f: Parrott & Smith, 1984; g: Taylor & Wiseman, 1987; h: Den Nijs & Stephenson, 1988; i: Sala et al., 1989; j: Wagenvoort et al., 1990; k: Van Santen et al., 1986; l: Van Santen, 1988; m: Maceira, 1990.

The occurrence of 2n pollen in alfalfa has been found to be controled by a single recessive gene (McCoy, 1982). In red clover, a single gene was responsible for the occurrence of 2n pollen and from 2 to 6 genes appear to be involved in the frequency of 2n gametes (Parrott & Smith, 1986a). Because a simple genetic control has been found for the production of 2n gametes which also shows a high degree of heritability, it has become feasible to increase 2n gamete frequency by recurrent selection e.g. in red clover (Parrott & Smith, 1986a) and alfalfa (Veronesi et al., 1989).

Tetraploid progeny from unilateral polyploidization (2x-4x and 4x-2x crosses) have been obtained in several legumes or grasses to widen the genetic base and further maximize heterozygosity and thus increase yield and persistence. For instance, in Dactylis several 4x progeny from 2x-4x crosses have shown favourable performance for many traits (relative to the 4x parents derived from natural tetraploid cultivars), thus indicating that yield improvement in cultivated Dactylis is still possible. However, as stressed by Casler & Hugessen (1988) and Van Santen & Casler (1990), selection at both the diploid and tetraploid levels will be necessary in Dactylis to achieve these goals. Such a selection implies that a broad genetic background, including numerous diploid individuals which would be able to produce high 2n gametes, is available. Unilateral polyploidization has also been used to produce polyploid hybrids in several fodder crops (Singh et al., 1990), namely in Trifolium ssp. (Parrott & Smith, 1986b) and Lotus (Negri & Veronesi, 1989). Up to now tetraploids from bilateral polyploidization (2x-2x crosses) have been obtained although at a low frequency in Trifolium pratense (Parrott et al., 1985; Parrott & Smith, 1986a) and Dactylis glomerata (Maceira, 1990 and unpublished data).

Conclusion

Diplogametes (2n gametes) are produced naturally and can be easily screened in many fodder crop. More particularly, when they are derived by FDR or an equivalent process, they possess a good potential for maximizing heterozygosity. Furthermore, their use in either unilateral or bilateral sexual polyploidization constitutes a very promising method for fodder legumes and grasses insofar as genetically broad initial diploid material is available. The use of somatic chromosome doubling is thus perhaps best reserved for specific purposes, such as obtaining artificial autopolyploids from diploid crops when suchs polyploids do not occur naturally.

References

Bingham, E.T., 1980. Maximizing heterozygosity in autopolyploids. In: W.H. Lewis (Ed.): Polyploidy: biological relevance. Plenum Press, New York. p. 471-489.

Bingham, E.T. & T.J. McCoy, 1979. Cultivated Alfalfa at the diploid level: origin, reproductive stability, and yield of seed and forage. Crop Sci. 19 : 97-100.

Casler, M.D. & P.M. Hugessen, 1988. Performance of tetraploid progeny derived from 2x-4x intersubspecific crosses in Dactylis glomerata L. Genome 30 : 591-596.

Demarly, Y., 1963. Génétique des tétraploides et amélioration des plantes. Ann. Amélior. Plant. 13 : 307-400.

Den Nijs, A.P.M. & A.G. Stephenson, 1988. Potential of unreduced pollen for breeding tetraploid perenial ryegrass. In: M. Cresti, P. Gori & E. Paeini (Ed.): Sexual reproduction in higher plants. Springer-Verlag, Berlin. p. 131-136.

Dunbier, M.W. & E.T. Bingham, 1975. Maximum heterozygosity in Alfalfa : results using haploid-derived autotetraploids. Crop Sci. 15 : 527-531.

Gallais, A. & P. Guy, 1970. Use of heterozis in breeding autotetraploids. Eucarpia fodder crop section meeting rep., Lusignan, France, p.105-118.

Hermsen, J.G.Th., 1984. Mechanisms and genetic implications of 2n gamete formation. Iowa State J. Res. 58 : 421-434.

Jansen, R.C. & A.P.M. Den Nijs, 1990. The statistical analysis of 2n and 4n pollen formation in Lolium perenne using pollen diameters. Eucarpia, Fodder crop section meeting report, Wageningen, The Netherlands.

Lumaret, R., 1984. The role of polyploidy in the adaptive significance of polymorphism at the GOT1 locus in the Dactylis glomerata complex. Heredity 52 : 153-169.

Lumaret, R., N.O. Maceira, A. De Haan & J. Delay, 1989. Screening of 2n gametes to produce new tetraploid material from diploids in Dactylis glomerata L. Proc. XVI Internat. Grassland Congress, Nice, France. p. 279.

Maceira, N.O., 1990. Signification évolutive de la polyploïdie: mécanismes et conséquences. Exemple de Dactylis glomerata L. (Graminacées). Thèse. Univ. Sciences & Techniques du Languedoc, Montpellier, France. 123 p.

McCoy T.J., 1982. The inheritance of 2n pollen formation in diploid alfalfa Medicago sativa. Can. J. Genet. Cytol. 24: 315- 323.

McCoy T.J. & D.E. Rowe, 1986. Single cross alfalfa (Medicago sativa L.) hybrids produced via 2n gametes and somatic chromosome doubling: experimental and theoretical comparisons. Theor. Appl. Genet. 72: 80-83.

McCoy T.J. & K. Walker, 1984. Alfalfa. In: P.V. Ammirato, D.A. Evans, W.R. Sharp & Y. Yamada (Ed.): Handbook of plant cell culture, 3. Crop species. MacMillan, New York, p. 171-192.

Negri, V. & F. Veronesi, 1989. Evidence for the existence of 2n gametes in Lotus tenuis Wald. et Kit. (2n=4X=24). Theor. Appl. Genet. 78: 400-404.

Parrott, W.A. & R.R. Smith, 1984. Production of 2n pollen in red clover. Crop Sci. 24: 469-472.

Parrott, W.A. & R.R. Smith, 1986a. Recurrent selection for 2n pollen formation in red clover. Crop Sci. 26: 1132-1135.

Parrott, W.A. & R.R. Smith, 1986b. Evidence for the existence of endosperm balance number in the true clovers (Trifolium ssp.). Can. J. Genet. Cytol. 28: 581-586.
Parrott, W.A., R.R. Smith & M.M. Smith, 1985. Bilateral sexual tetraploidization in red clover. Can. J. Genet. Cytol. 27: 64-68.
Pfeiffer, T.W. & E.T. Bingham, 1983. Abnormal meiosis in alfalfa, Medicago sativa : cytology of 2N egg and 4N pollen formation. Can. J. Genet. Cytol. 25: 107-112.
Pfeiffer, T.W. & E.T. Bingham, 1984. Comparisons of alfalfa somaclonal and sexual derivatives from the same genetic source. Theor. Appl. Genet. 67: 263-266.
Quiros, C.F., 1982. Tetrasomic segregation for multiple alleles in alfalfa. Genetics 101: 117-127.
Sala, C.A., E.L. Camadro, M.T. Salabury & A.O. Mendiburu, 1989. Cytological mechanisms of 2n pollen formation and unilateral sexual polyploidization in Lolium. Euphytica 43: 1-6.
Singh, A.K., J.P. Moss & J. Smartt, 1990. Ploidy manipulations for interspecific gene transfer. Advances in Agronomy 43: 199-240.
Tavoletti, S., A. Mariani & F. Veronesi, 1989. Analisi citologica della macrosporogenesi per l'individuazione dei meccanismi responsabili della produzione di gameti 2n in Medicago ssp. Rep. 33 convegno della Soc. Italiana di Genetica Agraria, Porto Conte, Alghero, Italy. p. 97-98.
Taylor, N.L. & E.O. Wiseman, 1985. Methodology and breeding of tetraploid red clover. Proc. XV IGC, Kyoto, Japan. p.244-245.
Taylor, N.L. & E.O. Wiseman, 1987. Triploids and tetraploids from 4x-2x crosses in red clover. Crop Sci. 27: 14-18.
Taylor, N.L., M.K. Anderson, K.H. Quesenberry & L. Watson, 1976. Doubling the chromosome number of Trifolium species using nitrous oxide. Crop Sci. 16: 516-518.
Teoule, E., 1983. Somatic hybridization between Medicago sativa L. and Medicago falcata L. C.R. Acad. Sci. Paris, Ser. III 297: 13-16.
Tomekpe, K. & R. Lumaret, 1991. Association between quantitative traits and allozyme heterozygosity in a tetrasomic species : Dactylis glomerata L. Evolution (in press).
Van Santen, E., 1988. Germplasm transfer in Dactylis. Ph. D. diss. Univ. of Wisconsin-Madison (diss. abstr. 88).
Van Santen, E., P.M. Hugessen & M.D. Casler, 1986. Tetraploid progeny from interploidy crosses in Dactylis glomerata L. ASA Spec. Pub. 48: 189.
Van Santen, E. & M.D. Casler, 1990. Forage quality and yield in 4x progeny from interploidy crosses in Dactylis L. Crop Sci. 30: 35-39.
Veronesi, F., A. Mariani & E.T. Bingham, 1986. Unreduced gametes in diploid Medicago and their importance in alfalfa breeding. Theor. Appl. Genet. 72: 37-41.
Veronesi, F., A. Mariani & S. Tavoletti, 1988. Screening for 2n gamete producers in diploid species of genus Medicago. Genet. Agr. 42: 187-200.
Veronesi, F., A. Mariani & S. Tavoletti, 1989. Selection for meiotic mutants (2n gamete producers) in diploid species of the fenus Medicago. XII Eucarpia Congress, Göttingen, Germany meeting Rep.
Veronesi, F., S. Tavoletti & A. Mariani, 1990. Identification of 2n and 4n gamete producers in an experimental population of diploid Medicago. J. Genet. & Breed. 44: 143-148.
Vorsa, N. & E.T. Bingham, 1979. Cytology of 2n pollen formation in diploid alfalfa, Medicago sativa. Can. J. Genet. Cytol. 21: 525-530.
Wagenvoort, M., de Vries van Hulter, G.D. Winkelhorst & A.P.M. den Nijs, 1990. Cytological mechanisms responsible for 2n pollen production and unilateral sexual polyploidization in perennial rye grass (Lolium perenne) Eucarpia, Fodder crop section meeting Rep., Wageningen, the Netherlands.

REGISTRATION AND TESTING OF VARIETIES - FUTURE DEVELOPMENTS

M.S. Camlin and A.C. McMichael

D.A.N.I., Plant Testing Station, Crossnacreevy, N. Ireland

Summary

With fodder crops such as grasses and clovers, several unique problems face registration and testing authorities. Varieties of these predominantly out-breeding crops are populations of different genotypes kept within prescribed descriptions by careful selection, maintenance and isolation during multiplication. Most useful distinguishing characteristics are continuously expressed within the populations with the result that varieties, in general, cannot be recognised through unique features.

The first problem which therefore arises is that breeders are seldom able to supply registration authorities with detailed information at the time of application, as is the case with many other crops. Determination of distinctness, uniformity and stability thus begins from a position of comparative ignorance and, because of genotype/environment interactions, takes several years to complete, involving measurement, recording and statistical manipulation of large volumes of data. In major species such as the ryegrasses, the increasing numbers of varieties in reference collections have added to the problems. With over 250 different perennial ryegrass varieties currently on EC and OECD lists and similar numbers of new varieties currently under test, it is surprising that, to date, distinctness is still achieved for most varieties with relative ease.

This paper discusses some of the new methods now being used by registration and testing authorities to meet the above problems. The future potential for increased use of more sophisticated techniques such as colour measurement, biochemical tests, electrophoresis and assessment of stress resistance is also examined. It is finally also suggested that, in future, testing authorities will have to give more attention to the use of special tests to investigate specific claims made by breeders.

Keywords: grass, clover, varieties, registration, testing

Introduction

With the out-breeding grasses, registration and testing authorities face several problems which, though in themselves not unique, combine to make the determination of distinctness of new varieties much more difficult than is the case with other crops. Adding to the problem is the fact that the number of varieties registered, particularly in the ryegrasses, has increased through the successful efforts of plant breeders. In the UK the potential future problem for both registration and breeding was recognised in the early 1980's. Although considerable advances had been made with statistical analysis, Weatherup (1980), field testing methods had remained largely unchanged since the introduction of methodology developed by Hawkins (1958). A research programme, was commenced at the Plant Testing Station, Crossnacreevy in 1982 to develop an integrated system for DUS testing of herbage varieties. The programme is now nearing completion and this paper attempts to give a preliminary view of the various components considered to be of importance within an integrated testing system.

Strategies in an Integrated DUS Programme

It was considered at the outset of the project that there were five main strategies which required to be examined and developed within an integrated programme:-

(1) Additional characters
(2) Automated data capture
(3) Improved statistical analysis
(4) Use of special tests
(5) New technologies

(1) Additional characters

During the project, approximately 60 potential new characters were investigated in detail for their discriminating power, correlations, uniformity implications and cost effectiveness. One of the main problems in trying to introduce new characters is the high level of correlation between certain types of characters, for example between plant height in spring, at heading and at full maturity or between leaf length and leaf width. This means that there is a "diminishing returns" situation with respect to the number of distinctness separations achieved by any group of characters. This is best illustrated by examination of the minimal character set as suggested by Weatherup (1980). The minimal character set for diploid perennial ryegrass grown at the Plant Testing Station, Crossnacreevy in 1990 is given in Table 1.

Table 1. Minimal character set - diploid perennial ryegrass.

UPOV CODE	CHARACTER	PAIRS SEPARATED ('000)	CUMULATIVE SEPARATIONS
8	HEADING DATE	25	24760
9	HEIGHT EE	18	2740
13	EAR LENGTH	16	862
	PLANT WIDTH	16	220
	GLUME LENGTH	15	197
11	LEAF WIDTH	17	117
14	SPIKELET NO.	14	83
10	LEAF LENGTH	17	55
2	PLANT ANGLE	8	43
	SPIKLET LENGTH	15	27
7	SPRING HEIGHT	17	18
12	STEM LENGTH	16	12
	PLANT HEIGHT	13	8
	INTERNODE LENGTH	12	3
		TOTAL	29245

It can be seen that all achievable separations, some 29,000, are obtained using 14 out of 20 measured characters but, more importantly, 99% of these separations are obtained using the first 6 characters alone. Heading date is by far the most effective character with almost 25,000 separations. However, while many other characters achieve between 15-18,000 separations their cumulative effect amounts to only some 3000 additional separations. Because of duplicate separations, many achieved by highly correlated characters, effectiveness of characters is thus progressively decreased.

There is a dilemma for testing authorities in providing an economic and effective testing system while trying to give breeders a fair chance to have their varieties shown distinct. The last 10 separations in Table 1, achieved by plant height and internode length have, in hindsight, been very costly to achieve, yet to the breeders involved they may be of vital importance for varietal distinctness. Also, because of genotype/environment interactions there is unfortunately no means of predicting exactly which characters will be important for a given set of varieties or years.

There is also the problem that any new character used in a routine manner across the whole variety collection is something of a "double edged sword". The advantages of extra distinctness opportunities for some varieties must be weighted against the extra uniformity hurdle imposed by the character on the full range of candidate varieties.

The compromise must be to design a set of characters which will effectively screen the variety collection and provide a cost effective proportion of "first-time" distinct decisions, leaving the remainder of problem cases to be examined further in row plots and special tests. For example, for the ryegrasses, it is considered that, with some minor amendments, the character set listed in the recently revised UPOV guideline, represents such a fair compromise.

(2) Automated data capture

This is an area where, with the advent of portable and robust data-loggers, the overall efficiency of the field trials system has been greatly increased in recent years. Data-acqusition programmes can be modified to provide for sequential, plant-by-plant recording or for recording of pre-selected individual plants. The most up-to-date data loggers can now also provide prompts to operators, identifying plants requiring to be examined for ear emergence on any particular day.

Data-loggers have also improved the accuracy of field recording, thanks to built-in range-checking systems. These plant-by-plant checks in the field are supplemented by back-up procedures in P.C's and mainframes to identify outlier plants within a variety, due for example to virus, which can, if appropriate, be deleted from the data-base before analysis.

One less obvious advantage of data-loggers is the speed with which the mainframe data matrix can be completed and analytical procedures commenced once field recording is finished. This now means that similar pairs of varieties can be identified in Mid-August, allowing sufficient time for row plots to be established in parallel with the subsequent years single spaced plants. The opportunity provided to "eyeball" varieties with potential distinctness problems in side-by-side comparisons as early as the second year of testing has been one of the most important and unforseen benefits of automated data capture.

New developments such as image analysis are under active investigation with other crops. The grass plant does not lend itself easily to such technologies but some research with seed-head characters is in progress at Crossnacreevy. An equally interesting and more recent development has been the development of field-based (as opposed to PC linked) voice recognition systems such as the MARCONI TALKMAN. With the same data capture features and power as key-board data-loggers these open the way for "hands -free" data logging and may allow us to dispense with the traditional two-person measurement and logging operation.

With the large amounts of information being gathered from field trials there is little doubt that efficient and accurate data measurement and collection will continue to be a key element of an integrated DUS testing programme.

(3) Improved statistical analysis

This is undoubtedly the area where most significant advances have been made in grass DUS testing over the past ten years. We have come a long way from the rigid within-years system where two differences at P=0.01 over three years (2 x 1%) was used to indicate varietal distinctness. The first improvement was the introduction of the 't-score' system which allowed more flexibility for consistent differences to be summed across years. This was the precursor to a much more sophisticated and elegant system, the Combined Over-Years (COY) analysis first proposed by Patterson and Weatherup (1984) and now adopted by UPOV as the standard statistical means of distinctness. This provides a single over years criterion by which to judge varietal distinctness.

From Table 2 it can be seen that, over the 1988-90 test period under the old 2 x 1% system, approximately 56% of candidate varieties in the UK would have been rejected or required special tests to show distinctness. However, using the recently agreed standard of the COY 1% analysis, only 21% of varieties remain indistinct or require further examination in special tests or row plots.

Table 2. Discrimation in diploid perennial ryegrasses (1988-90).

Statistical Criterion	Distinct Varieties	Non-distinct Varieties
2 x 1%	35	45
t-score	49	31
COY 1%	63	17
COY 5%	80	0

Undoubtedly, without the continued improvement and development of statistical methods the DUS testing systems would by now have experienced major difficulties with distinctness, especially for the more popular crops. We do however need to sound a note of caution. Statistical procedures, by their nature, involve risks and we must be careful not to erode standards to the extent that the system is providing insufficient protection for breeders. When one recognises that breeders are frequently submitting sister-lines and that many are also using very similar parental material, the apparently very favourable result using COY 5% (Table 2) is surely a step too far. There are also implications for "minimum distances" and a balance must be struck between a benevolent system for applicant varieties and adequate protection for established market leaders. Statisticians and technical officers within UPOV are now agreed that COY 1% provides a satisfactory balance for distinctness in single spaced plants, to be backed up if necessary by row plots and special tests for problem cases.

Research has already commenced on a multivariate (across characters) distinctness criterion. This has possibilities but we must ensure that the basic principles of, firstly, linking varietal uniformity in any character to distinctness and, secondly, providing meaningful biological descriptions for varietal differences are retained, in addition to maintaining an awareness of the implications for "minimum distances".

Despite these cautionary remarks there is little doubt that sophisticated statistical procedures which will allow identification of <u>real</u> varietal differences will continue in future to be central to DUS testing in the cross-fertilized grasses and clovers.

(4) <u>Special Tests</u>

As plant breeders continue to search for new variation and unique varietal features it is important that the registration and testing systems should not be so rigid that they can not permit the introduction of new characters and technologies to investigate these developments. Nevertheless, many special tests are expensive to conduct and there is a need to first explore all the possibilities for distinctness offered by standard characters. This, if not providing clear distinctness, should at least reduce the number of problem varieties requiring examination in special tests. The early identification of similar varieties and use of row plots to examine these is considered a very cost-effective extension to the first stage of screening of the whole variety collection in single spaced plant trials.

In order not to delay decisions on varieties it is proposed that special tests should in future be undertaken in the third test year if required by the breeder. However, these should perhaps be conducted at the breeder's direct expense on the principle that those requiring sophisticated and expensive technology should meet the relevant costs rather than fees for all breeders being increased across the board.

There can possibly never be a completely definitive set of DUS characters except perhaps in the context of the preliminary screening process. The flexible integration of single spaced plants, rows/plots and special tests, if necessary employing new technologies, must be practiced to allow the examination and recognition of unique and novel varietal features.

(5) New technologies

New technologies are likely in future to form an important part of the integrated DUS testing programme. However, because of their relative expense it is anticipated that there use in routine testing work will not be extensive. Their role is likely to be most effective in discriminating between similar pairs of varities identified after routine screening in single spaced plants and row plots, and in investigation of special claims made by breeders.

Three technologies are currently under investigation within the research programme at Crossnacreevy; Colour measurement, Chlorophyll fluorescence and Electrophoresis.

Colour Measurement

One of the most frequent claims made by breeders is of varietal differences in colour, especially with the amenity grass species and varieties. It is however important to differentiate between true leaf colour differences and other differences caused by disease, management or fertility problems. Colour is not easily assessed by eye in single spaced plant trials and so row plots provide an excellent means of screening for colour differences. However, there are shortcomings in that differences cannot be quantified and the uniformity of varieties cannot be properly estimated.

The introduction of colour measuring instruments, for example the MINOLTA CHROMA METER solves both problems. It provides standardised and accurate measurement of colour parameters X,Y,Z (red, green, blue), L, a, b (lightness, red/green, yellow/blue) and L, C, H (lightness, chroma, hue) which can be related to true colour. The instrument is also field-portable so that it can be used in a semi-routine fashion. Research at Crossnacreevy was originally conducted in the early stages of the programme, Hamill & Camlin (1984), and since 1988, all similar varieties identified from single spaced plants and rows have been routinely screened for colour differences.

Chlorophyll Fluorescence

This is an elegant technique which allows plant material to be screened for stress tolerence, often at a very early stage before symptoms become evident. Features which can and have been examined in various crops were reviewed by McMichael et al. (1989) and include cold, heat, drought, herbicide, chemical and disease stress. However, with most stress testing, whether or not chlorophyll fluorescence is used, the main problem is in sample presentation and stress application. Herbicide or chemical stresses are relatively easy to apply and can usually be achieved in hydroponic culture, Harris & Camlin (1988). Other stresses are less simple and in particular the difficulties with stress application for winterhardiness determination in grasses are currently under investigation. It is hoped that methods can be developed for this and other stress testing to allow this technique to play a more active role in future investigation of special claims by breeders.

Electrophoresis

Much has been said and written about this technique and its possible application for DUS testing in herbage crops over the past ten years, for example, Hayward and McAdam (1977), Gilliland _et al_. (1982), Hayward _et al_. (1983), Wright _et al_. (1983) Gardner _et al_. (1986). There is little doubt that the techniques, whether using seed and leaf proteins or isoenzymes, are highly discriminating and can provide stable varietal descriptors. It is not the intention in this paper to discuss in detail the merits or otherwise of introduction of electrophoresis into registration procedures. Suffice to say that there are still concerns about implications for varietal uniformity and "minimum distances". Several Testing Authorities have extensive data-bases available and much research has been conducted. The question of introduction of electrophoresis in grasses is a matter for breeders to resolve among themselves and then present their views to Testing Authorities and to UPOV. It is however clear that electrophoresis can only be an additional technique within any testing programme. Because electrophoretic characters are not necessarily associated with morphological uniformity (or distinctness), field based testing will continue to be required.

With all new technologies careful consideration of methods of utilization is required so that better varietal discrimination can be achieved _without_ reducing "minimum distances" and eroding the protection of Plant Breeder's Rights.

Conclusion

There are a number of new developments which may have potential in DUS testing of grasses and clovers. These may include new morphological characters , new methods of data recording and analysis and new technologies for special tests. It does however need to be emphasised that the main requirement is for an efficient and cost effective testing system. To achieve this there is a logical progression from field-based screening in single spaced plants to identify similar varieties, through inexpensive "eyeball" examination in row plots to special tests, if necessary using sophisticated techniques. The less expensive routine methods must be fully explored and problem areas identified clearly before the more costly special tests are undertaken.

At Crossnacreevy an integrated testing scheme is now in place along the lines indicated in Table 3.

Table 3. An integrated DUS testing scheme for grasses.

YEAR		TEST/TRIALS	REPORT
1		SINGLE SPACED PLANTS	PRELIMINARY DESCRIPTIONS
2	(A)	SINGLE SPACED PLANTS	FINAL REPORTS*
	(B)	ROW PLOT COMPARISONS	INTERIM REPORTS
3	(A)	SINGLE SPACED PLANTS	FINAL REPORTS
	(B)	ROW PLOT COMPARISONS	
	(C)	SPECIAL TESTS	

* APPROXIMATELY 60% PASSED DUS AT YEAR 2

The UK Herbage DUS Research Programme will terminate in 1991. It is hoped that further refinements will be added to the above scheme once the final report on the programme is produced. However the integration of single spaced plants, row plots and special tests will undoubtedly form the framework for future DUS testing of grasses in the UK.

Acknowledgement

The authors would like to thank Mr Brian Waters, Dr Colin Weatherup and their staff for assistance and encouragement during the conduct of this research programme, and MAFF and DANI for financial support.

References

Gilliland, T.J., M.S. Camlin & C.E. Wright, 1982. Evaluation of phosphoglucoisomerase allozyme electrophoresis for the identification and registration of cultivars of perennial ryegrass (Lolium perenne). Seed Science and Technology 10: 415-430.

Gardiner, S.E., M.B. Forde & C.R. Slack, 1986. Grass cultivar identification by sodium dodecylsulphate polyacrylamide gel electrophoresis. New Zealand Journal of Agricultural Research 29: 193-206.

Hamill, M. & M.S. Camlin, 1984. The measurement of leaf colour in grasses. Journal of Agricultural Science, Cambridge 103: 387-393.

Harris, M. & M.S. Camlin, 1988. Chlorophyll fluorescence as a rapid test for reaction to urea herbicides in winter wheat. Journal of Agricultural Science, Cambridge 110: 627-632.

Hawkins, R.P., 1958. The classification of the strains (varieties) of herbage plants. Journal of the National Institute of Agricultural Botany 9: 434-449.

Hayward, M.D. & N.S. McAdam, 1977. Isoenzyme polymorphism as a measure of distinctness and stability in cultivars of Lolium perenne. Zeitschrift fur Pflanzensuchtung 79: 9-68.

Hayward, M.D. & N.S. McAdam, 1983. Interpretation and application of isozyme techniques for variety discrimination in grasses. Proceedings of an International Seed Testing Association Symposium on Biochemical Tests for Cultivar Identification, National Institute of Agricultural Botany, Cambridge p. 78-84.

McMichael, A.C., M. Harris & M.S. Camlin, 1989. Application of chlorophyll fluorescence kinetics in the study of varietal reaction to stress. Plant Varieties and Seeds 2: 45-51.
Patterson, H.D. & S.T.C. Weatherup, 1984. Statistical criteria for distinctness between varieties of herbage crops. Journal of Agricultural Science, Cambridge 102: 59-68.
Weatherup, S.T.C., 1980. Statistical procedures for distinctness, uniformity and stability trials. Journal of Agricultural Science, Cambridge 94: 31-46.
Wright, C.E., T.J. Gilliland & M.S. Camlin, 1983. Electrophoresis: Implications for Plant Breeders' Rights. Proceedings of an International Seed Testing Association Symposium on Biochemical Tests for Cultivar Identification, National Institute of Agricultural Botany, Cambridge p. 41-50

OPPORTUNITIES FOR BIOTECHNOLOGY IN CLOVER BREEDING

K Judith Webb and Ian Rhodes

Institute for Grassland and Environmental Research, Welsh Plant Breeding Station, Aberystwyth, Wales, UK

Summary

Conventional breeding techniques, coupled with extensive genetic resources and an understanding of clover's interaction with a range of biotic factors, have produced new, reliably productive varieties. Breeders have identified characters which are not present in the existing gene pool of white clover but which could improve the crop even further. Techniques of tissue culture, such as somatic hybridization, somaclonal variation and genetic transformation, will all have their part to play, not only in transferring new genes but also in increasing the understanding of the regulation and expression of foreign genes in legumes. Such novel genes have already been transferred into several herbage legumes, including white clover.
Key words: white clover, breeding programme, tissue culture, genetic transformation, novel genes.

CURRENT STATE OF CLOVER BREEDING

The widespread use of white clover has been inhibited by its reputation for unreliability, low yield, lack of persistency under intensive grazing and propensity to cause bloat in cattle. These problems are being overcome by genetic improvement.

The success of white clover in a sward is dependent on its interactions with the companion grass, grazing animal, Rhizobium and its response to climatic and edaphic factors. Seed production is also partly dependent on interactions with pollinating insects. At Aberystwyth, our approach has been to develop an understanding of the physiological and ecological basis of these interactions, enabling the development of morpho-physiological selection criteria and models which have already been used in producing new varieties.

The current objectives of the programme are to improve: 1) yield and reliability through a) grass/clover compatibility b) tolerance to stress, particularly the stress of temperature extremes, drought and waterlogging and c) resistance to pests and diseases 2) yield and persistency under intensive grazing 3) nitrogen fixation and thus total sward yield 4) seed yield potential and harvestability of seed.

1. Yield and reliability

Large differences in compatibility exist between varieties. These differences may be of a general nature with, for example, certain grass varieties being more aggressive to a range of clover varieties. However, specific compatible relationships are occasionally found between pairs of grasses and clovers, such that they yield either poorly or well together.

Studies on the basis of compatibility are leading to the development of selection criteria to improve both spatial and temporal compatibility (Rhodes et al., 1988) and ultimately the development of grass and clover varieties designed to be grown together.

The loss of stolon through winter kill and slow growth of white clover in spring, place it at a competitive disadvantage with the companion grass. We have shown that annual clover yield in a mixture is closely related to the quantity and quality of stolon surviving the winter (Collins et al., 1990). By achieving a combination of extreme cold hardiness and good low temperature growth, we have produced varieties of white clover which possess a better general compatibility with grass (Rhodes et al., 1989).

With regard to disease and pest resistance, good progress has been made in improving resistance to the major problems of Sclerotinia and Ditylenchus dipsaci.

2. Yield and persistency under intensive grazing

A strong interaction exists between clover variety and type of defoliation (Evans and Williams, 1987). However, a major development in breeding has been the breaking of the negative relationship between yield (leaf and petiole) and amount of stolon (and thus, persistency under grazing). Thus it has proved possible to increase the yield of small-leaved types, without sacrifice in persistency under grazing, and conversely increase the persistency of large-leaved types without decrease in yield (Rhodes and Webb, 1989).

3. Improved nitrogen fixation and total sward yield

Some clover varieties appear to 'support' increased yields of grass, without sacrifice in clover yield (Evans et al., 1990). Several new varieties possessing this feature are now undergoing National List testing (Rhodes and Webb, 1989). The precise mechanisms of the effect are not generally understood and are the subject of further investigation.

4. Seed yield potential

Seed yield potential has been increased without sacrifice in agronomic performance and potential exists for further improvement. In addition, pollination and harvestability of seed may be improved by increasing length and strength of the peduncle as in the potential variety Ac 3715 (Rhodes and Webb, 1989). Manipulation of spread of flowering also offers possibilities for harvesting more of the seed produced.

Many of the problems inherent in the use of white clover have been or are being solved by conventional breeding technology. The potential for improvement remains considerable because of the our better understanding of the basis of variation in yield and extensive genetic resources. These improved varieties will become available to the farmer over the next five years. Following this and over a time span of the next fifteen years, we are likely to see the introduction of compatible packages of grasses and clovers.

WHAT NOVEL GENES CAN BIOTECHNOLOGY BRING TO WHITE CLOVER?

Techniques of tissue culture can be used both to manipulate existing genes and to introduce new genes into plants. Genes controlling desirable traits occur in sexually incompatible species of clover, in unrelated plants, in bacteria or in viruses. Gene transfer makes these genes available to the breeder. In legumes, several approaches to gene manipulation or transfer can be adopted (Nisbet and Webb, 1990; Rhodes and Webb, in press) but the final choice depends on the objective of the investigation and the genetic control of the character involved.

A wide range of targets have been identified for study. These include the relationship between the presence of tannins in plants and the occurrence of bloat in grazing animals (McLeod, 1974), the effect of improved fodder quality on wool growth in sheep (White, 1988; Rogers, 1990) and resistance to pests and diseases (White, 1988).

1. Nutritional quality

Protein is digested mainly in the rumen of sheep and cows. During digestion, complex interactions occur which can affect the value of the forage.

Bloat

Certain crops, including white clover, occasionally cause bloat in animals. This problem causes concern and has inhibited the widespread use of clover in dairy farming. Bloat results from the rapid release of gases and the production of a stable protein foam in the animal's rumen during digestion. Prophylactic treatments are available but the fundamental question of why only certain crops cause bloat is unresolved. In non-bloating legumes, the build-up of the foam may be prevented by either of two mechanisms: the thickness of the cell wall may slow the rate of digestion, or the tannins found in their leaves and stems may precipitate proteins which have been released.

Tannins are found in several legumes, including several species of Trifolium, allowing the option of gene transfer to white clover either by sexual hybridization, followed by embryo rescue, or by somatic hybridization. In addition, genes for key enzymes in the secondary metabolic pathway leading to the production of tannins have already been cloned (Robbins et al., 1990). Genetic manipulation of legumes both with and without tannins using these genes should result in plants with altered levels of tannin production. Such plants could then be used in studies on digestion and cause of bloat in animals.

High quality proteins

Wool growth in sheep can be stimulated by proteins which contain the sulphur-rich amino acids, cysteine and methionine (Rogers, 1990). To be effective, these high quality proteins must pass into the intestine relatively undigested. Two pea seed proteins - pea albumen 1 (PA1) and vicilin - have been identified. Of these PA1 resists digestion in the rumen but is easily digested in the intestine. Fusion of the cloned genes with a promoter which directs expression to leaves, rather than seeds, could result in a new high quality white clover producing a protein from pea seeds in its foliage (White, 1988, Rogers, 1990).

2. Resistance to pests and diseases

The overall loss of white clover in the field to pests and diseases is difficult to assess. Incorporation of resistance to various pathological organisms will help provide an idea of the scale of these losses.

Resistance to insects

Various insects are pests of clover, affecting the vegetative parts or the root system. Toxins (Höfte and Whiteley, 1989) produced by the bacterium *Bacillus thuringiensis* offer one approach to insect control. Different strains of the bacterium produce toxins (Bt toxins) which are active against different insect groups. These toxins are activated only when solubilised and cleaved in the gut of the insect.

In white clover, the larvae of the beetle, *Sitona lepidus*, preferentially attack root nodules. Production and accumulation of active toxin in root nodules could prevent this damage. The toxin can be targeted to the nodules by constructing strains of *Rhizobium* containing the toxin gene (Skøt et al, 1990) or by introducing the gene, under the control of a nodule-specific promoter, into the legume itself.

Another approach utilizes proteinase inhibitors which are produced by some plants in response to wounding. These proteins inhibit proteinase activity in the gut of the feeding insect, so preventing digestion of proteins. A modified potato proteinase inhibitor II gene is being introduced into white clover (White 1988).

Resistance to viral infection

Several viruses infect white clover, but the severity of the problem remains unknown. White clover mosaic virus is endemic in some areas. It produces no visible symptoms, which makes accurate assessment of its effect on yield in the field very difficult.

Plants inoculated with mild strains of viruses can confer cross-protection following subsequent infection of the plant. Transfer of cDNA coding for the coat protein of a virus can provide similar cross-protection. cDNA cloned from white clover mosaic virus is to be transferred into white clover (White, 1988).

APPROACHES AND DEVELOPMENTS SO FAR

White clover

Central to all techniques of gene transfer is the ability of different parts of the plant, such as immature, fertilised ovules, leaves or protoplasts, to regenerate into plants *in vitro*. The ease of regeneration varies considerably between species, varieties and even between individual plants. For some herbage legumes, regeneration systems are well established, but in white clover reliable and routine regeneration of shoots and plants is limited to certain genotypes (Bond and Webb, 1989). While this restricts the choice of starting material, it will not hamper the application of genetic manipulation in white clover.

Hybrids between sexually incompatible Trifolium species have been produced in various laboratories. These hybrids combine characters from both parental species (Webb and Rhodes, in press). Transformation systems are available for white clover, and these have resulted in the introduction of a selectable marker gene (coding for resistance to the antibiotic kanamycin) into clover shoots and its expression in those shoots (White and Greenwood, 1987). Other genes, including a selectable marker gene coding for hygromycin resistance and an easily visualised reporter gene, ß-glucuronidase (GUS) (Webb et al., 1990) and a pea lectin gene (Diaz et al., 1989) have been transferred to clover roots. These developments permit studies of gene expression under different promoters in white clover and can allow experimental systems to be established and some hypotheses to be tested. For example, the pea lectin gene could affect the plant's ability to fix nitrogen since it influences the host range of the nitrogen fixing bacterium, Rhizobium.

Other herbage legumes

Many of the scientific principles can equally be tested using other, more easily manipulated legumes, such as Medicago sativa and Lotus corniculatus. Hybrid plants, somaclonal variants and genetically transformed plants have been produced in both of these species (Rhodes and Webb, in press). Several genes have been introduced into M.sativa, including selectable marker genes and genes coding for the seed proteins, pea albumen 1 and vicilin (Ford, 1988; Rogers, 1990). Both of these genes were active in M.sativa, but further work is needed to improve gene expression.

L.corniculatus has been used in studies of various aspects of nitrogen fixation. Genes, such as soybean leghaemoglobin (Stougaard et al., 1987) and glutamine synthetase (Forde et al., 1989) have been introduced and their expression studied. At the Cell Manipulation Group, IGER-WPBS, we are interested in the expression and regulation of several genes in L.corniculatus, including the reporter gene, GUS, and genes involved in tannin biosynthesis and in production of Bt toxin. Other work involves a study of the inheritance and continued expression of GUS in the progeny of transgenic plants. Information gained from these studies will ultimately be applied to white clover.

CONCLUSIONS

New varieties which have been designed to eliminate some of the more fundamental problems discussed in this paper are now being entered in National List Trials and will be available to the farmer within 5 to 7 years. Following this, and over a time span of up to 15 years, we will see the introduction of packages of compatible grasses and clovers bred specifically for mixture growth and ensuring reliable and higher total mixture yield.

Beyond this period, we hope to see the benefits of new technologies introducing new characters, some highlighted in this paper, into what is a reliable and consistently productive pasture component.

References

Bond JE & KJ Webb, 1989. Regeneration and analysis of plants from stolon segments of Trifolium repens (white clover). Plant Sci 61: 119-125.

Collins RP, MJ Glendining & I Rhodes, in press. The relationships between stolon characteristics, winter survival and annual yields in white clover (Trifolium repens L.). Grass and Forage Sci.

Diaz CL, LS Melchers, PJJ Hooykass, BJJ Lugtenberg & JW Kijne, 1990. Root lectin as a determinant of host-plant specificity in the Rhizobium-legume symbiosis. Nature 338: 579-581.

Evans DR & TA Williams, 1987. The effect of cutting and grazing managements on dry matter yield in white clover varieties (Trifolium repens) when grown with S23 perennial ryegrass. Grass and Forage Sci 42: 153-159.

Evans DR, TA Williams & SA Mason, 1990. Contribution of white clover varieties to total sward production under typical farm management. Grass and Forage Sci 45: 129-134.

Ford J, 1988. Sheep will grow woolier on a bio-engineered diet. New Scientist 117 (10 March) p24.

Forde BG, HM Day, JF Turton, S Wen-jun, JV Cullimore & JE Oliver, 1989. Two glutamine synthetase genes from Phaseolus vulgaris L. display contrasting developmental and spatial patterns of expression in transgenic Lotus corniculatus plants. The Plant Cell 1: 391-401.

Höfte H & HR Whiteley, 1989. Insecticidal crystal proteins of Bacillus thuringiensis. Microbiol Rev 53: 242-255.

McLeod MN, 1974. Plant tannins - their role in forage quality. Nutrition Abstr Rev 44: 803-815.

Nisbet GS & KJ Webb, 1990. Transformation in legumes. In: Ed YPS Bajaj. Biotechnology in Agriculture and Forestry Vol 10. Legumes and Oilseed Crops I 38-48.

Rhodes I & KJ Webb, 1989. White clover varieties for the future. In: The attractions of forage legumes. British Grassland Soc. Hurley. 20pp.

Rhodes I & KJ Webb, in press. Progress in white clover breeding. J Agric Sci.

Rhodes I, RP Collins, DR Evans & MJ Glendining, 1989. Breeding reliable white clover for low input pastures. Proc XVIth Intl Grassland Congr, Nice 315-316.

Robbins MP, TE Evans, TR Carron & P Morris, 1990. Gene expression related to condensed tannin biosynthesis in forage legumes. J Exp Bot, SEB Annual Meeting Abstracts, Warwick. P5-12.

Rogers GE, 1990. Improvement of wool production through genetic engineering. Tibtech 8: 6-11.

Skøt L, SP Harrison, A Nath, LR Mytton & BC Clifford, 1990. Expression of insecticidal activity in Rhizobium containing the δ-endotoxin gene cloned from Bacillus thuringiensis subsp tenebrionis. Plant & Soil 127: 285-295.

Stougaard J TE Petersen, KA Marcker, 1987. Expression of a complete soybean leghaemoglobin gene in root nodules of transgenic Lotus corniculatus. Proc Natl Acad Sci 84: 5754-5757.

Webb KJ, S Jones and M Gibbs, 1990. Use of Agrobacterium rhizogenes binary vector to transform herbage legumes. VIIth Intl Cong Plant Tissue Culture 24-29 June.

White DWR, 1988. Use of cell and molecular genetic manipulation to improve pasture plants. Proc N Z Grassland Assoc 49: 67-72.

White DWR and D Greenwood, 1987. Transformation of the forage legume Trifolium repens L. using binary Agrobacterium vectors. Plant Mol Biol 8: 461-469.

IN VITRO SELECTION IN FORAGE CROPS

U.K. POSSELT

Landessaatzuchtanstalt, Universität Hohenheim, Postfach 70 05 62,
D-7000 Suttgart 70, Federal Republic of Germany

Summary

In vitro selection takes advantage of the genetic variability released in vitro. However, a working regeneration system is an undispensable prerequisite. Besides stress tolerance (i.e. salinity, heat , drought) disease resistance is of major interest to plant breeders. The selection techniques available are described and the current literature in forage crops is reviewed.
The problems of in vitro selection for resistance to pathogens by means of toxins will be discussed according to our research work on snow mould resistance in perennial ryegrass.
Keywords: forage crops, in vitro-selection, selective agents

Introduction

Improved disease resistance is a major goal in the breeding programs of many plants, including forage crops. Tissue culture techniques offer a variety of new ways to identify, select, and transfer genes involved in disease resistance.
In vitro selection (IVS) is an appealing strategy: large numbers of single cells or cell clumbs (with little variation in cell type) can be grown in a small space under control of environment and nutritional factors (EARLE, 1983). IVS takes advantage of the genetic variability released in vitro (BUIATTI, 1989). This phenomenon is called somaclonal variation and could be demonstrated in various crops, including forages. In Lotus corniculatus and Medicago sativa somaclonal variation for various characters was demonstrated by ARCIONI et al. (1986) and NAGARAJAN & WALTON (1989), respectively.
According to the type of selection or selective agent, the resultant variants can be categorized as amino acid resistant, analog resistant, antibiotic resistant, auxotrophic, chilling resistant, herbicide resistant, pathotoxin resistant or salt tolerant (BUIATTI, 1989). Examples of IVS in forage crops are summarised in table 1.
However, it has to be stressed, that a working regeneration system is an undispensable prerequisite of IVS. Though in vitro systems have been established for most of the forage crops, regeneration is still the bottle neck in the Gramineae.
According to EARLE (1983) many steps may be involved in IVS for disease resitance using a toxin as selective agent:

A Generalized *scheme for in vitro selction* (after EARLE, 1983, modified)

1. Preparation of appropriate cultured material
2. Mutagenesis (optional)
3. Exposition of cultures to a selective agent and selection of resistant variants
4. Regeneration
5. Test of regenerants for toxin and pathogen resistance
6. Test of sexual and asexual progenies
7. Introduction of resistant material in the breeding process

Strategies for disease resistance selection.

In disease resistance breeding both sides, the host and the pathogen have to be considered. Depending on the type of breeding material (lines or populations) and the type of pathogen (obligate or facultative parasite) the host-pathogen-interaction (HPI) can be very complex. There are various signals and mechanisms within the HPI-system, which have been discussed by LAMB et al. (1989).

Table 1 Examples of IVS-experiments

disease resistance

Species	Pathogen	Selective Agent	References
Medicago	F.oxysporum	culture filtrate	HARTMANN et al.
Medicago	F.oxysporum	culture filtrate	ARCIONI et al.
Medicago	Phytophthora	mycelium	MILLER et al.
Agrostis	Rhizoctonia	HPI-system	TOMASO & KRANS

herbicide and stress tolerance

Species	Selective Agent	References
Lotus	2,4-D	SWANSON & TOMES
Trifolium	2,4-D	TAYLOR et al.
Medicago	NaCl	McCOY
Medicago	Al	PARROT & BOUTON
Agropyron	NaCl	SHI DECHENG
Agrostis	Zn, Cu	WU & ANTANOV

Cell and tissue culture provide several levels on which selection for resistance can be applied:

1. Whole plant or plant parts
2. Testing in vitro regenerants in vivo
3. In vitro selection using selective agents
3.1. During regeneration
3.2. Callus cultures
3.3. Single cells
3.3.1. Liquid medium
Cell suspension, protoplast
isolated micro- and macrospores
3.3.2. Solid medium
anthers and ovaries
plated microcalli from 3.3.1.

In conventional breeding programs selection for disease resistance is commonly practiced on whole plants. The same is true for testing in vitro regenerants in vivo. The latter has been applied on Medicago (Verticillium) by LATUNDE-DADA & LUCAS (1983) and on Cynodon (Helminthosporium and insect resistance) by CROUGHAN (1989). Though unwanted somaclones (i. e. with undesired morphology) can easily be detected, large numbers of plants have to be grown and observed. Because no particular selection pressure is applied in vitro, the efficiency of this type of selection is questionable.

Though detached leave methods may include in vitro techniques they are not in vitro selection in the strict sense. The same is true for pathogen elimination by means of in vitro techniques.
In vitro selection using a selective agent can be practised during regeneration, in callus cultures (common practice) or on single cells, the latter being the most sensitive.
Whether selection is applied on diploid or haploid cells or tissue, and in liquid or solid medium, depends mainly on the techniques available in the crop of interest.

Types of selective agents

The use of living pathogens as selective agents has the virtue of directness, eliminating concerns about the relevance of a toxin to a disease. However fungal pathogens may overgrow plant cells on the culture medium, resulting in a confusing system in which results are difficult to interpret (EARLE, 1983). MILLER et al. (1984) used living mycelium to screen callus from Medicago for Phytophthora resistance. TOMASO-PETERSON & KRANS (1990) developed a co-culture system (HPIS) which avoids physical contact with the living pathogen, but allows diffusion of metabolites through a membrane (such systems are commercially available).
A sequential interaction system (double layer technique) was described by LEPOIVRE et al. (1986), where mycelium growth is stopped by fungicide treatment. In a second step culture medium is placed as a second layer on top of the pathogen medium. It is expected, that fungal metabolites will move into the culture medium and influence the development of the cultured cells.

The use of nonliving selective agents which can be readily sterilised and incorporated into a nutrient medium eliminates at least some of the difficulties of dealing with the pathogens themselves (EARLE, 1983).
Culture filtrates of Fusarium oxysporum have been used to screen callus cultures of Medicago (ARCIONI et al., 1987; HARTMAN et al., 1984). The latter group could demonstrate that virulent cultures produced toxins whereas avirulent cultures did not.

Pathotoxins are grouped into host-specific toxins and nonspecific ones. A host-specific toxin is a pathogen produced metabolite with the same specifity for plant material as the pathogen itself (EARLE, 1983). Unfortunately, no such toxins are known for most diseases.
Therefore, the application of nonspecific toxins may be justified, though only poor correlations between in vitro and in vivo response might be expected. Much more information about the role of toxins in HPI, both in vivo and in vitro, are needed.

Even with progress in this area, tissue culture techniques will not displace conventional field approaches to breeding for disease resistance. However, they may contribute significantly to the agricultural improvement of some crops (EARLE, 1983).

Improvement of snow mould resistance in Lolium perenne

Fusarium nivale (syn. Microdochium nivale) is the main pathogen which causes snow mould. Together with F. culmorum and F. graminearum it is responsible for various diseases in grasses and cereals. In the latter, contamination of harvested kernels with toxins is a severe problem in some years. Because most Fusarium species are known toxin producers, we assumed the same will be true for F. nivale. A conventional screening technique for resistance against snow mould in Lolium perenne on the whole plant level had been established some years ago (POSSELT, unpublished).

In 1987 we started to use tissue culture techniques in Lolium perenne. At first, organ culture with lateral buds was established to maintain donor plants in vitro. Secondly, callus culture from various explants was worked out and a technique based on mature embryos could be established (see SCHMIDT & POSSELT, this volume).

Originally it was planned to use culture filtrate from a set of F. nivale isolates as the selective agent. Both, filtration and sterilization posed several technical difficulties, and additionally, nothing was known about the fungal metabolites. The double layer technique, which was considered as an alternative had to be modified as no fungicides against F. nivale were available. To stop the growth of mycelium autoclaving at 121 °C for 20 minutes had to be applied at least twice instead. It is assumed, that this procedure released compounds hasardeous to callus cultures from the fungal substrate as the calli turned necrotic within one week after subculture.

Because several of the above mentioned Fusarium species produce Deoxynivalenol (DON) this trichothecene was used in purified form obtained by a commercial supplier. However, it has to be mentioned that DON is too expensive for large scale IVS experiments.
A number of isolates from various Fusarium species were screened for their potential of in vitro DON formation, to obtain this toxin in the quantities needed for in vitro selection of callus cultures. As one result of this work our group was able to proof that isolates of F. nivale as well have the ability to produce thrichothecenes.

To relate the toxin to the pathogen (i.e. the disease) germinating seeds of Lolium perenne were exposed to various concentrations of DON in a biotest, showing a clearcut dosage response. The coleoptiles of most seedlings showed symptoms similar to those from pathogen contaminated seeds.

Our first selection experiments with callus cultures using DON as selective agent showed that low concentrations (10^{-5} mol) had a promoting effect on the proliferation of the cultures but their regenerating ability was lost. We assume that relatively high concentrations (in the range of 10^{-4} to 10^{-3} mol) for a short time (5 to 10 days) are needed to induce a sufficient selection pressure without loss of regeneration ability. Such experiments are currently on the way.

References

Arcioni, S., M. Pezzotti, & F. Damiani, 1987.
In vitro selection of alfalfa plant resistance to Fusarium oxysporum f.sp. medicaginis. TAG 74: 700–705.

Arcioni, S., D. Mariotti, F. Damiani, & Pezzotti, M., 1986.
Somaclonal variation in Lotus corniculatus L. in: Genetic Manipulation in Plant Breeding. pp 581–584. Eucarpia, Berlin.

Buiatti, M. 1989.
Use of cell and tissue cultures for mutation breeding.
Vortr. Pflanzenzüchtung 16:179–200.
Croughan, T. P., S. J. Stavarek & D. W. Rains, 1978.
Selection of a NaCl tolerant line of cultured Alfalfa cells.
Crop. Sci. 18: 959–963.
Croughan, S. S. 1989. Forage crop improvement through biotechnology.
XVI. Int. Grassl. Congr., Nice, France.
Decheng, Shi; Xi Ti & Zhu Tingcheng, 1989.
Somatic Embryogenesis from mature embryo of tall wheatgrass (Agropyron elongatum) and effects of NaCl on induction and growth of callus from its different explants. XVI. Int. Grassl. Congr. Nice, France.
Earle, E. 1983.
The use of phytotoxins for in vitro selection of disease-resistant plants. Cell & tissue culture techniques for cereal improvement. Beijing, pp 265–278.
Hartmann, C. L., T. J. McCoy & T. R. Knous, 1984.
Selection of alfalfa (Medicago sativa) cell lines and regeneration of plants resistant to the toxin(s) produced by Fusarium oxysporum f. sp. medicaginis. Plant Sci. Lett., 34: 183–194.
Lamb, Chr. J., M. A. Lawton, M. Dron & R. A. Dixon, 1989.
Signals and transduction mechanisms for activation of plant defenses against microbial attack. Cell, 56: 215–224.
Latunde-Dada, A. O. & J. A. Lucas, 1983.
Somaclonal variation and reaction to verticillium wilt in Medicago sativa L. plants regenerated from protoplast.
Plant Sci. Lett. 32: 205–211.
Lepoivre, P., J. Viseur, K. Duhem, & N. Carels, 1986.
Double-layer culture technique as a tool for the selection of calluses resistant to toxic material from plant pathogenic fungi.
In: J. Semal (Ed.) Somaclonal variation and crop improvement.
Martinus Nijhoff Publ., pp 45–53.
McCoy, T. J. 1987.
Tissue culture evaluation of NaCl tolerance in Medicago species: cellular versus whole plant response. Plant Cell Reports 6: 31–34.
Miller S. A., L. C. Davidse & D. P. Maxwell. 1984.
Expression of genetic susceptibility, host resistance, and nonhost resistance in alfalfa callus tissue inoculated with phytophthora megasperma. Phytopatholgy 74: 345–348.
Nagarajan, P. & P. D. Walton. 1989.
Evaluation of R1 alfalfa somaclones for herbage yield and morphological characteristics. Plant Breeding 102: 333–337.
Parrot, W. A. & J. H. Bouton. 1990.
Aluminium tolerance in Alfalfa as expressed in tissue culture.
Crop Sci. 30: 387–389.
Swanson, E. B. & D. T. Tomes. 1980.
In vitro responses of tolerant and susceptible lines of Lotus corniculatus L. to 2,4-D. Crop Sci. 20: 792–795.
Tomaso-Peterson, M. & J. V. Krans. 1990.
Evaluation of a new in vitro cell selection technique.
Crop Sci. 30: 226–229.
Taylor, S. G., D. J. Shillig & K. H. Quesenberry. 1989.
In vitro selection for 2,4-D tolerance in red clover. TAG 78: 265–270.
Wu, L. & J. Antonov. 1978.
Zinc and copper tolerance of Agrostis stolonifera in tissue culture.
Am. J. Bot. 65: 268–271.

REGENERATION FROM PROTOPLASTS OF PERENNIAL RYEGRASS; PROGRESS AND APPLICATIONS

J. Creemers-Molenaar and J.P.M. Loeffen

Barenbrug Holland BV, Oosterhout, The Netherlands

Summary

Procedures are described for improved plant regeneration from suspension cultures and protoplasts in perennial ryegrass (Lolium perenne L.). Cold storage of regeneration competent suspension cultures prolonged the regeneration period of these cultures. Substitution of part or all of the sucrose by maltose in the suspension culture medium did not affect the regeneration frequency of these cultures. From protoplasts that were isolated from a 13-week old suspension culture up to 334 green plants/$3.5x10^5$ protoplasts have been regenerated. In perennial ryegrass intraspecific protoplast fusion by the PEG-method was superior to electro-fusion.
Keywords: Lolium perenne L., protoplasts, protoplast fusion.

Introduction

The new tools of genetic engineering bring into focus the possibility of transforming grasses with e.g. disease resistance genes. Furthermore, techniques like somatic hybridization and cybridization offer the possibility to combine alien genomes or make new nucleus-cytoplasm combinations. In monocotyledonous species like cereals and grasses, progress towards the development of efficient transformation and somatic cybridization and hybridization techniques has been hampered by several obstacles (Potrykus, 1990):
1) Although many dicots have been transformed by using Agrobacterium species as a vector, until now no Agrobacterium transformed plants have been recovered from any monocot species. 2) Protoplasts are suitable for transformation by direct DNA-uptake. However, plant regeneration from protoplasts is still difficult to achieve in most cereals and grasses.

Work has been carried out to improve methods for suspension culture initiation, plant regeneration from protoplasts and protoplast fusion in Lolium perenne L. The results will be discussed in relation to the applicability of techniques for genetic engineering.

Results and discussion

1 Suspension culture initiation

A fast method to establish suspension cultures in perennial ryegrass has been developed by Dalton (1988). The cultures are initiated by transferring 30-40 mature seed-derived embryos directly into liquid medium with a high 2,4-D concentration. The cultures are continuously kept on a rotary shaker to facilitate the release of small callus pieces. Established suspension cultures can be obtained as soon as 8 weeks after culture initiation and are maintained by weekly subculture

into fresh medium. Upon plating on solid regeneration medium young cultures regenerate up to 200 green shoots per gram fresh weight callus. Between cultures of one variety much variation is observed with respect to regeneration and growth characteristics.
Suspension cultures in perennial ryegrass can also be initiated from morphogenic callus. Explants of immature inflorescences are cultured on solid medium with 2,4-D and morphogenic callus is selectively subcultured several times on the same medium. After 2-3 months embryogenic callus is chopped into small pieces and transferred to liquid medium for suspension culture initiation. The advantage above the former method is that each culture is derived from only one genotype, making it possible to select plants (genotypes) with a good response for callus induction and suspension culture initiation.

Established suspension cultures begin to yield sufficient quantities of protoplasts 12-16 weeks after initiation. However, most suspension cultures have lost the ability to regenerate plants within 20-25 weeks after initiation. Consequently the period during which regeneration-competent protoplasts can be obtained is very short.

Several ways have been and are being explored to retain the regeneration capacity of Lolium suspension cultures longer and to make the successful establishment of suspension cultures more reproducible:
-**Carbohydrates**. Substitution of sucrose by maltose improved the anther response of anther cultures in Lolium perenne L. (Bante et al., 1990). In cultures of alfalfa maltose improved the morphology and conversion to plantlets of somatic embryos (Strickland et al., 1987). However, replacement of part or all of the sucrose by maltose in the suspension culture medium of several Lolium lines did not prolong the regeneration period (Fig. 1).
-**Cold storage**. The effect of storage on the regeneration frequency of fast growing, regeneration competent suspension cultures of Lolium at 4°C for 6 weeks without shaking is shown in Figure 2. The regeneration competence of plated suspension culture Lp25 increased after cold storage and this higher level of regeneration was maintained several weeks after transfer to standard culture conditions. Similar results were obtained with suspension cultures of other varieties. This good response of Lolium suspension cultures to storage at low temperature permits prolonged use of the cultures and is time-saving with respect to subculture regimes.
-**Genotype selection**. Meristems of in vitro grown regenerants from protoplasts (Fig. 3a) and immature inflorescences of field-grown regenerants from protoplasts and suspension cultures (Fig. 3b) have been used for callus induction for the initiation of suspension cultures. The aim is to select genotypes which proliferate callus types that reproducably yield fast growing suspension cultures with small callus pieces and a high regeneration frequency. Meristems from in vitro grown regenerants from protoplasts provided excellent explant material for callus induction. Data for the amenability of these callus cultures for the establishment of suspension cultures are not yet available.

2 Protoplast isolation, culture and regeneration

Regeneration of plants from suspension-derived protoplasts of Lolium has been described in detail by Dalton (1988) and Creemers-Molenaar et al. (1989). In a typical experiment $3,5x10^5$ protoplasts are plated in a

3 cm Petri dish. Of these 0.1 to 1% will form microcalli and from these calli fertile plants have been regenerated (Fig. 3b). Table 1 summarizes the results of the frequency of plant regeneration from protoplasts in relation to suspension culture age. Thirteen weeks after suspension culture initiation less than 0.01% of the isolated protoplasts formed microcalli. However, up to 334 green plants were obtained from protoplast-derived microcalli from one dish. When the suspension culture had aged only one week further, the regeneration frequency decreased to only 14 shoots per dish of plated protoplasts. To improve plant regeneration from protoplasts of Lolium, the percentage of division and morphogenesis competent protoplasts from young suspension cultures must be further increased. The addition of antioxidants to the protoplast isolation and culture medium appears to be a promising direction of research (Creemers-Molenaar and van Oort, 1990).

Table 1. Plant regeneration from protoplasts of Lolium perenne L. in relation to suspension culture age.

suspension age (weeks)	plating[1)] efficiency	number of shoots[2)] green	albino
13	<0.01 (2)	334 (2)	19
14	0.04 (6)	14 (6)	16
16	0.02 (3)	12 (7)	1
17	0.4 (3)	6 (7)	2
21	0.8 (1)	6 (3)	2
24	0.4 (2)	0 (12)	0

1) Percentage of microcalli after 4 weeks of culture. ( )= the number of dishes counted.
2) The number of green and albino shoots was determined per 3.5×10^5 plated protoplasts. ( )= number of plates counted.

3 Protoplast fusion

With the aim of transferring mitochondrion encoded traits within Lolium species, asymmetric protoplast fusions have been carried out. To inactivate the cytoplasm the recipient protoplasts have been treated with iodoacetamide, while the donor protoplasts have been treated with γ irradiation to destroy the nuclear DNA. Protoplast fusion has been attempted using electro-fusion and PEG-fusion techniques (poly ethylene glycol). In Lolium, electro-fusion of suspension protoplasts yields fusion frequencies of up to 5%, but the treatment appears to be so destructive that after fusion no microcalli are formed. Treatment with PEG results in similar fusion frequencies, while only a minor decrease of the plating efficiency of protoplasts is observed. From asymmetric fusion experiments calli have been obtained, which will be characterized by Southern blot analysis using mitochondrial and nuclear probes.

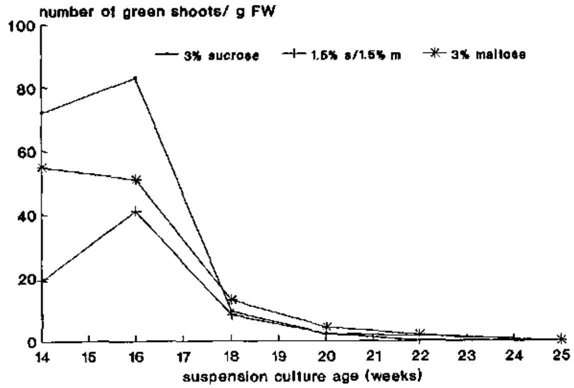


Fig. 1. The effect of maltose on plant regeneration from suspension culture Lp25 of Lolium perenne L. Suspension cultures were maintained in standard MS medium as described previously (Creemers-Molenaar et al. 1989). From week 14 the cultures were maintained in the standard MS5 medium supplemented with 1) 3% sucrose, 2) 1.5% sucrose and 1.5% maltose 3) 3% maltose. After plating on regeneration medium the average number of shoots/g FW was determined from triplicate samples from 4 Erlenmeyers.

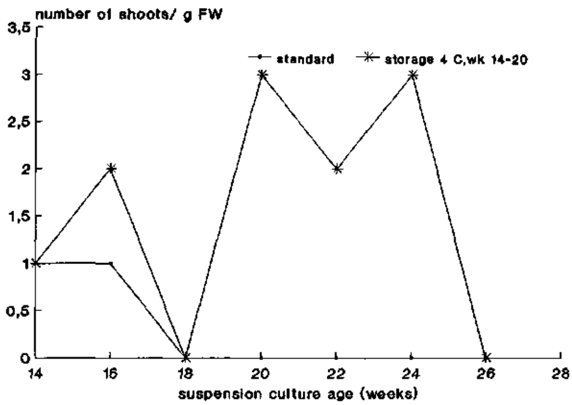


Fig. 2. The effect of cold storage on plant regeneration from suspension culture Lp25 of Lolium perenne L. 14 weeks after initiation one culture was divided over 8 Erlenmeyer flasks. Four cultures were stored at 4°C and returned to standard culture conditions at 25°C, 110 rpm. after 6 weeks. The other 4 cultures were kept under standard conditions continuously. Every 2 weeks the 8 cultures were plated on regeneration medium as described previously (Creemers-Molenaar et al. 1989). The average number of shoots/g FW was determined from triplicate samples from 4 Erlenmeyers.

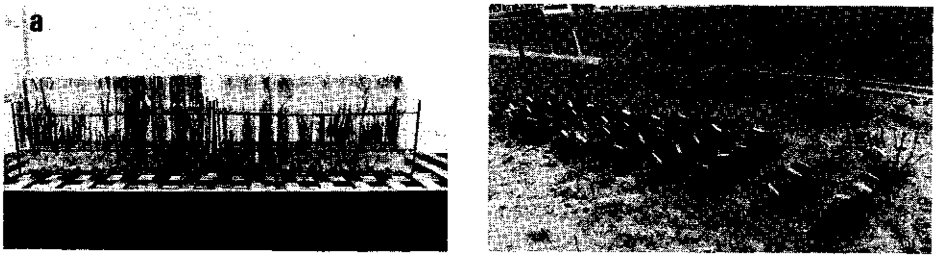


Fig. 3 a-b. Plants regenerated from suspension culture-derived protoplasts of Lolium perenne. a in vitro grown plants b after transfer to the field.

Conclusion

Recently, successful transformation of cereals has been obtained by the method of direct DNA-uptake by protoplasts and by bombardment of suspension cells with DNA-coated particles (Potrykus, 1990). The availability of procedures for plant regeneration from suspension cultures and protoplasts in Lolium perenne makes both methods of genetic transformation feasable for this important forage grass species. Furthermore, when plants can be regenerated from cybrid and hybrid calli, protoplast fusion can become an additional tool for the genetic manipulation of perennial ryegrass.

Acknowledgements

This work has been carried out in close collaboration with the Center for Plant Breeding Research (CPO), Wageningen.

References

Bante, I., T. Sonke, R.F. Tandler, A.M. van den Bruel and E.M. Meijer, 1990. Anther culture of Lolium perenne L. and Lolium multiflorum L. Acta Bot. Neerl. 39(1):103.

Creemers-Molenaar, J.P. Van der Valk and J.P.M. Loeffen, 1989. Plant regeneration from suspension cultures and protoplasts of Lolium perenne L. Plant Sci. 63:167-176.

Creemers-Molenaar, J. and Y. Van Oort, 1990. Antioxidants influence plating efficiency and microcallus growth of protoplasts in Lolium perenne L. In: H.J.J. Nijkamp, L.H.W. van der Plas and J. van Aartrijk (Eds.): Progress in plant cellular and molecular biology, Proceedings VIIth International Congress on Plant Tissue and Cell Culture. Kluwer academic publishers, Amsterdam. p.44-49.

Dalton, S.J., 1988. Plant regeneration from cell suspension protoplasts of Festuca arundinacea Schreb. (tall fescue) and Lolium perenne L. (perennial ryegrass). J. Plant Physiol. 132:170-175.

Potrykus, I., 1990. Gene transfer to cereals: an assessment. Bio/Technology 9:535-542.

Strickland, S.G., Nichol, J.W., McCall, C.M. and Stuart, D.A., 1987. Effect of carbohydrate source on alfalfa somatic embryogenesis. Plant Sci. 48:113-121.

HAPLOIDS IN GRASSES BASED ON KNOWLEDGE FROM CEREALS

Andersen S.B., A. Olesen, N. Halberg, and S. Madsen

Royal Veterinary and Agricultural University, Copenhagen, Denmark[1]

Summary

From the literature on anther culture in cereals it is evident that the growing conditions for the donor plants are of some importance for the results. A more pronounced effect, however, comes from genes in the parent plants. This has lead to the extensive use of especially high responding model genotypes during the development of the haploid methods for the cereals. Albinism is a curious phenomenon in anther culture of barley, wheat and rize, but practically without existence in dicots like the brassicas. The ability to form high fractions of green regenerants is to a high extent determined by genes inherited on the chromosomed in wheat and barley. Newly established systems for haploid induction in anther culture for several of the fodder grasses are based on the methods and growth media adopted from the cereal anther cultures. Reproducible results are still restricted to rare genotypes, mainly because of genotype dependence for formation of embryos from the cultured anthers and genetical determination of the ability to produce green plants. Current work attempting to identify genotypes with high anther response in these species either through screening of large number of different clones or through genetic recombination of the genes controlling the anther culture response has two main purposes: High responding model clones may be used to confer the anther culture ability to ordinary breeding material of the species through various types of backcross approaches build into the traditional breeding programmes. The high responding types are valuable experimental material during adaptation of the anther culture methods to the species in order to obtain generally high response with most genotypes.
Keywords: haploids, Anther culture, Grasses, Genotype effect

Introduction

Homozygotization of plant material is an important step in many plant breeding programmes to improve the genetic gain during selection and to achieve genetic stability during muliplication of selected material. Theoretically, completely homozygous plants can be obtained through chromosome doubled haploids which can be produced in a large number of plant species through a variety of different methods. The production of androgenetic haploids from microspores by means of anther or microspore culture followed by spontaneous or chemically induced chromosome doubling is the method most generally applicable to a wide number of species (Dunwell 1986).

Experience from cereal anther culture

For hexaploid wheat (Triticum aestivum L.) and barley (Hordeum vulgare L.) the first microspore derived plants from anther culture were reported by Ouyang et al (1973) and Picard and de Buyser (1973) in wheat and Clapham (1973) in barley. In spite of great efforts spent on the anther culture technique in these species the improvements to the methods remained sparse for almost a decade. This was mainly due to what may be named "the vicious circle" of newly established anther cultures. When the methods are still virtually unadapted to a species, formation of embryos and plants are too sporadic to permit safe conclusions regarding the effects of changes to the media and treatments. This leads to a continued low and fluctuating response.

1. Present address: Dept of Agricultural Science, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK 1871, Copenhagen, Denmark

Progress with the culturing methods in wheat and barley began to appear when particularly, responsive genotypes were identified. Among those from wheat: 'Ciano', and 'Orofen' (Ouyang et al 1983), and in barley: 'Sabarlis' (Huang and Sunderland 1982), and 'Igri' (Köhler and Wenzel 1985). The generally high and reproducible response of such "model" varieties enabled systematic experiments, from which sound conclusions could be drawn to improve the culture system. For a detailed review of the literature concerning improvements of donor plant conditions, pretreatment of plant material, culturing media and methods see Picard et al (1990) for wheat and Xu (1990) for barley. Particularly in the case of barley anther culture the optimization of cold pretreatments for the spikes (Huang and Sunderland 1982), the introduction of 'positioning' of the anthers on the media surface (Hunter 1985, Shannon 1985), and the exchange of sucrose with maltose in the media (Hunter 1987) have improved the anther culture results to an extent where many genotypes of barley will produce sufficient microspore derived plants for breeding purposes (Kuhlmann and Foroughi-Wehr 1989). Similar universal improvements to wheat anther culture still remains to be seen, though optimal temperature regimes during the culture (Ouyang et al 1983) and spraying the donor plants with gametocides (Picard et al 1987) are generally believed to improve the results. The wheat anther culture, however, contrary to the one of barley, has been successful enough to produce seweral new varieties, among those 'Florin' (de Buyser et al 1987) and cv. Jinghua No. 1 (Hu Daofen 1986).

The formation of high percentages of albino plants, which will not survive outside the in vitro culture, is a curious and serious problem in most anther cultures of cereals and grasses. The albino plants have been demonstrated to possess immature plastids, apparently blocked in various stages of development towards maturity (Clapham 1973, Sun et al 1974). Hybridization studies using DNA-probes have demonstrated deletions or alterations of part of the chloroplast genome in albino plants (Day and Ellis 1985). The extreme capacity of several of the widely used model varieties to produce high percentages of green plants in the anther culture, however, has been shown through the study of reciprocal crosses, to be determined by genes inherited on the chromosomes in wheat (Tuvesson et al 1989). Apart from these facts not much is known about the nature of the albino problem.

The successful identification and exploitation of high responding model varieties is the result of a strong genotypic effect on the ability to respond in anther culture. These genotypic effects have been investigated in a number of studies in both barley (Dunwell et al 1987, Powell 1988) and wheat (Lazar et al 1984, Deaton et al 1987) giving detailed information concerning the embryo formation. Regeneration and green plant formation could not be studied separately in these investigations due to the limited number of plants obtained. The total green plants/100 anthers is the product of the three basic response components: Embryo formation (embryos/100 anthers), regeneration (plants/100 embryos), and the percentage of green plants. Recently, it has been possible to study the three basic components separately for both wheat (Andersen et al 1987) and barley (Knudsen et al 1988). The studies have demonstrated that genetical control is important for embryo formation and the capacity to form green plants, while of little importance for regeneration of the microspore embryos into plants. Generally, the growth environments employed for the donor plants during these studies have had only small effects on the results compared to the effects of the genotype of the plant material.

Results with grasses

The attempts to produce haploids from microspores in grasses were started as early as those in the cereals (Clapham 1971), but considerably less efforts have been devoted to the grass species. The reports on successful plant formation from anther culture in grass species until 1987 have been summarized in table 1. The content is mostly pioneer work with very low percentages of response which could not permit systematic adaptation of the anther culture methods to the species. Using methods and media adapted from wheat anther culture, Olesen et al (1988) reported results from a screening experiment with 30 different genotypes of perennial ryegrass (Lolium perenne L.), where on average 19.2 plants were obtained per 100 anthers cultured. Though the majority of plants produced were albinos, three diploid ryegrass clones with a capacity to produce 1-3 green plants per 100 cultured anthers were identified.

Table 1. Reported experiments where plants were obtained through anther culture in grasses until 1987.

Festuca		
F. arundinacea	green plants	Kasperbauer et al (1980)
F. pratensis	albino plants	Rose et al (1987a,b)
Dactylis		
D. glomerata	green plants	Conger & McDonnell (1982)
Lolium-Festuca hybrids		
L. multiflorum x F. pratensis	green plants	Rose et al (1987a,b)
F. pratensis x L. multiflorum	green plants	Nitzsche & Wenzel (1977)
L. perenne x F. pratensis	green plants	Rose et al (1987a,b)
L. multiflorum x F. arundinacea	green plants	Nitzsche (1970)
Lolium		
L. multiflorum	albino plants	Clapham (1971)
	green plants	Niizeki (1977)
	green plants	Nitzsche & Wenzel (1977)
	green plants	Pagniez & Demarly (1979)
L. temulentum	albino plants	Rose et al (1987a,b)
L. perenne	albino plants	Stanis et al (1983)
	green plants	Stanis & Butenko (1984)

Since then several reports on green plant production in both Lolium perenne and Lolium multiflorum have appeared using other substrates and modified methods (Bante et al 1990) or modifications of the technique used for barley anther culture (Boppenmeier et al 1989). However, the general level of response even with the best ryegrass clones are still rather low (1-2 green plants/100 anthers). Though important information has been obtained concerning types and concentration of carbohydrate and concentration of growth substances for optimal embryo formation from the cultured anthers, conclusions regarding the total green plant production are still uncertain due to the low number of green plants obtained from such material.

The green plants obtained have enabled several investigations of their properties. Apparently, 50-60% of the anther derived plants from diploid ryegrasses are diploid, the remainder being haploids with a low fraction of polyploids in both L. perenne and L. multiflorum (Olesen et al 1988, Bante et al 1990). This level of spontaneous chromosome doubling in anther culture of diploid L. perenne was confirmed in the study of Hayward et al (1990). However, Boppenmeier et al (1989) observed a considerably lower frequency of spontaneously chromosome doubled plants of only 29 %. Plants derived from anther culture of tetraploid material of the same species consist of about half diploids and half tetraploids and some plants with higher ploidy (Bante et al 1990).

Several investigations of plants derived from anther culture of diploid material of L. perenne heterozygous in one or more isozyme loci have demonstrated the majority of plants to contain only one of the parental isozyme markers thus being either haploid or homozygously diploid (Olesen et al 1988, Hayward et al 1990). We investigated the isozyme patterns of 913 plants derived from anther culture of diploid L. perenne donor plants heterozygous in one or more of three unlinked isozyme loci (GPI/2, GOT/2, GOT/3). Only three heterozygous plants were found which showed the heterozygous pattern from their donor plants in both GPI/2 and GOT/2. These plants are thought to be the result of regeneration from unreduced microspores. In addition, 7 plants with both parental isozyme alleles but without a hybrid band were found. Such plants are thought to be chimeras formed through fusion of young microspore embryos during early development.

A characteristic feature of the anther culture process in ryegrass seems to be that genetic markers often show segregation of the alleles different from the 1:1 pattern expected according to Mendelian expectation. We studied the segregation of isozyme alleles among anther derived plants of diploid L. perenne and observed highly significant deviations from 1:1 ratios in 10 out of 18 cases. Some of the distorted segregations are presented in Table 1. Such deviations from a 1:1 ratio could be the result of environmental effects, lethal genes, or genes affecting the developmental rate during anther culture linked to the isozyme marker. Hayward et al (1990) have presented a good example of either lethals or major genes affecting anther culture response linked to the isozyme markers. Samples of anther derived plants were produced and analysed for two isozymes independently in two laboratories. The results showed a consistent selection of one allele of each isozyme from one of the donor clones and selection of the other allele from the second donor clone. The segregation could be explained by linked lethals or major genes affecting anther culture response linked in coupling phase in one donor clone and repulsion phase in the other clone. Whether these genes are linked lethals or linked genes affecting the development into green plants during the anther culture is not known. The selection taking place, however, will reduce the genetic variation among the doubled haploids. If the selection is mainly due to elimination of lethal or semi-lethal genetic factors in the parent material it could be an advantage for breeding purposes, because it will reduce the level of inbreeding depression expected in completely homozygous plants. If the distorted segregations are mainly the result of selection for genotypes with special capacity for in vitro growth the selection could be a disadvantage for breeding purposes, since such types may not be agronomically desirable.

Table 2. Distorted segregation of isozyme alleles among green (G) and albino (A) plants derived by anther culture from diploid parent plants of Lolium perenne heterozygous in the locus.

Isozyme GOT/3						
clone	175x255 No. 7		255x245 No. 14		255x175 No. 6	
allele	G	A	G	A	G	A
bb	0	0	24	28	3	21
cc	47	83	7	13	13	45
CHI^2	47.0***	83.0***	9.3**	5.5*	6.3*	8.7**

Isozyme GOT/2						
clone	255x245 No. 14		175x255 No. 9		255x175 No. 6	
allele	G	A	G	A	G	A
aa	2	3		34	3	8
bb	29	35		59	13	45
CHI^2	23.5***	26.9***		6.7**	6.3*	25.8***

*,**,***: significant at 5, 1, and 0.1 % level, respectively

Recently, we succeeded to construct very responsive genotypes of diploid Lolium perenne (Halberg et al 1990) through hybridization of three responsive clones identified previously (Olesen et al 1988). Among 55 hybrid clones tested for anther culture response in two environments used for the donor plants, 6 clones were identified with an anther culture response level superior to their parents. While the parents produced one green plant or less per 100 cultured anthers, these superior clones produced from 11 to 59 green plants/100 anthers. Variance components calculated from the analysis of variance for the three response components showed patterns of genetic and environmental determination very similar to those observed for wheat and barley. Genetical effects are predominant for embryo formation and the capacity to form green plants, accounting for 57 and 73 per cent of the total variation, respectively, while of less importance for regeneration, accounting for only 32 per cent of total variation. The two environments for the donor plants were of little importance for any of the three response components. Thus the 10-50 times improvement of the number of green plants/100 anthers in these superior clones were mainly established through improvements of the genetical capacity for embryo formation and the genetical capacity for green plant formation and the two traits were successfully combined.

We think the progress seen for L. perenne through identification of primary responsive clones in the species, followed by their hybridization and selection of recombined offspring with high anther culture response could also be obtained in several of the other grass species. Similar to what has been the case with the cereals these "model" genotypes will be valuable in grasses during future attempts to improve the culturing procedures and media. This is because the level of response of this material is high enough to allow safe conclusions to be drawn regarding the effects of various changes to the procedures. Thus with this type of material it should be possible to break the "vicious circle" of the grass anther culture. Contrary to the situation in the cereals such high responding clones could even be valuable in the breeding of grasses as a sort of "inducers", if they can confer the capacity for anther culture response to ordinary breeding material through hybridization. If the inducer types cannot be selected among adapted material with high agronomic performance one or a few cycles of backcrossing may overcome the problem. However, this "inducer" approach will only be generally useful if the capacity to respond in anther culture can be demonstrated to show a reasonable degree of dominance. Further for the approach to be useful the constraints on the choice of one parent of the breeding material must be counterbalanced by a considerable advantage of using such homozygous clones instead of traditional heterozygous plant material. Whether these two requirements can be met, still remains to be seen.

References

Andersen SB, Due IK, Olesen A (1987): The response of anther culture in a genetically wide material of winter wheat (Triticum aestivum L.). Plant Breed 99: 181-186

Bante I, Sonke T, Tandler RF, van den Bruel AMR, Meyer EM (1990): Anther culture of Lolium perenne and Lolium multiflorum. In: B.S. and R.S. Sangwan (eds) The impact of biotechnology in agriculture. International conference held in Amien, France 10-12 July 1989 (in press)

Boppenmeier J, Züchner S, Foroughi-Wehr B (1989): Haploid production from barley yellow dwarf virus resistant clones of Lolium. Plant Breed 103: 216-220

Buyser de J, Henry Y, Lonnet P, Hertzog R, Hespel A (1987) "Florin": A doubled haploid wheat variety developed by the anther culture method. Plant Breed 98: 53-56

Clapham D (1971): In vitro development of callus from the pollen of Lolium and Hordeum. Z Pflan zenzücht 65: 285-292

Clapham D (1973): Haploid Hordeum plants from anthers in vitro. Z Pflanzenzücht 69: 142-155

Conger BV, McDonnell (1982): Plantlet formation from panicle cultures of mature orchardgrass (Dactylis glomerata L.) plants. Proc. 5th intl.Cong.Plant Tissue and Cell Culture. Plant Tissue Culture. pp 523-525

Day A, Ellis THN (1985): Deleted forms of plastid DNA in albino plants from cereal anther culture. Curr Genet 9: 671-678

Deaton WR, Metz SG, Armstrong TA, Maxcia PN (1987): Genetic analysis of the anther-culture response of three spring wheat crosses. Theor Appl Genet 74: 334-338

Dunwell JM (1986): Pollen, ovule and embryo culture as tools in plant breeding. In: Withers LA, Alderson PG (eds) Plant tissue culture and its agricultural applications, Butterworths, London Boston Durban Singapore Sydney Toronto Wellington. pp 375-404

Dunwell JM, Francis RJ, Powell W (1987): Anther culture of Hordeum vulgare L.: A genetic study of microspore callus production and differentiation. Theor Appl Genet 74: 60-64

Halberg N, Olesen A, Tuvesson IKD, Andersen SB (1990): Genotypes of perennial ryegrass (Lolium perenne L.) with high anther-culture response through hybridization. Plant Breed (in press)

Hayward MD, Olesen A, Due IK, Jenkins R, Morris P (1990): Segregation of isozyme marker loci amongst androgenetic plants of Lolium perenne L. Plant Breed 104: 68-71

Huang B, Sunderland N (1982): Temperature-stress pretreatment in barley anther culture. Ann Bot (London) 49: 77-88

Hunter CP (1985) The effect of anther orientation on the production of microspore-derived embryoids and plants of Hordeum vulgare cv. Sabarlis. Plant Cell Rep 4: 267-268

Hunter CP (1987) Plant generation method. European Patent Application, Nr. 0 245 898 A2, 1-8

Hu Daofen (1986): Jinghua No. 1, a winter wheat derived from pollen sporephyte. In: Hu Han, Yang Hongyuan (eds) Haploids in higher plants in vitro. China Academic Publishers Beijing, Springer, Berlin Heidelberg New York Tokyo, pp 137-145

Kasperbauer MJ, Buckner RC, Springer WD (1980): Haploid plants by anther-panicle culture of tall fescue. Crop Sci 20: 103-107

Knudsen S, Due IK, Andersen SB (1989): Components of response in barley anther culture. Plant Breed 103: 241-246

Köhler F, Wenzel G (1985): Regeneration of isolated barley microspores in conditioned media and trials to characterize the responsible factor. J Plant Physiol 121: 181-191

Kuhlmann U, Foroughi-Wehr B (1989): Production of doubled haploid lines in frequencies sufficient for barley breeding programs. Plant Cell Reports 8: 78-81

Lazar MD, Baenziger PS, Schaeffer GW (1984): Combining abilities and heritability of callus formation and plantlet regeneration in wheat (Triticum aestivum L.) anther cultures. Theor Appl Genet 68: 131-134

Niizeki M (1977): Haploid, polyploid and aneuploid plants from cultured anthers and calluses in species of Nicotiana and forages crops. Journ. of the Faculty of Agriculture, Hokkaido University 58: 343-466

Nitzsche W (1970): Herstellung haploider Pflanzen aus Festuca-Lolium-Bastarden. Naturwissenschaften 57: 199-200

Nitzsche W, Wenzel G (1977): Haploids in Plant Breeding. Fortschritte der Pflanzenzüchtung 8: 46-48. Berlin, Hamburg: Verlag Paul Parey. Advances in Plant Breeding

Olesen A, Andersen SB, Due IK(1988): Anther culture response in perennial ryegrass (Lolium perenne L.). Plant Breed 101: 60-65

Ouyang JW, Hu H, Chuang CC, Tseng CC (1973): Induction of pollen plants from anthers of Triticum aestivum L. *cultured in vitro. Sci Sin 16: 79-95*

Ouyang JW, Zhou SM, Jia SE (1983): The response of anther culture to culture temperature in Triticum aestivum. Theor Appl Genet 66: 101-109

Pagniez M Demarly Y. (1979): Obtention d'individus androgénétiques par culture in vitro d'anthères de Ray-grass d'Italie (Lolium multiflorum Lam.). Ann Amélior Plantes 29: 631-637

Picard E, Buyser de J (1973): Obtention de plantules haplöides de Triticum aestivum L. à partir de cul tures d'anthères in vitro. CR Acad Sci Paris, Ser D 277: 1463-1466

Picard E, Hours C, Gregoire S Phan TH, Meunier JP (1987): Significant improvement of androgenetic haploid and doubled haploid induction from wheat plants treated with a chemical hybridization agent. Theor Appl Genet 74: 289-297

Picard E, Rode A, Doussinault G, Rousset M, Rives M (1990) Wheat (Triticum aestivum): In vitro production and utilization of doubled haploids. In Y.P.S. (ed) Biotechnology in Agriculture and Forestry, Vol 12 Haploids in Crop Improvement I. Springer-Verlag Berlin Heidelberg, pp 101-124

Powell W (1988): Diallel analysis of barley anther culture response. Genome 30: 152-157

Rose JB, Dunwell JM, Sunderland N (1987a): Anther culture of Lolium temulentum, Festuca pratensis and Lolium x Festuca hybrids. I. Influence of pretreatment, culture medium and culture incubation conditions on callus production and differentiation. Annals of Botany 60: 191-201

Rose JB, Dunwell JM Sunderland N (1987b): Anther culture of Lolium temulentum, Festuca pratensis and Lolium x Festuca hybrids. II. Anther and pollen development in vivo and in vitro. Annals of Botany 60: 203-214

Shannon PRM, Nicholson AE, Dunwell JM, Davies DR (1985): Effect of anther orientation on mi crospore-callus production in barley (Hordeum vulgare L.) Plant Cell Tissue Organ Culture 4: 271-280

Stanis VA, Butenko RG (1984): Developing viable haploid plants in anther culture of ryegrass. Doklady Biological Sciences 275: 249-251

Stanis VA, Slesaravichus AK, Kaleda VA (1983): [Regeneration of ryegrass and clover plants in tissue culture of different origin](in russian). Sel'Skokhozyaistvennaya Biologiya 5: 29-31

Sun CS, Wang CC, Chu CC (1974): The ultrastructure of plastids in the albino pollen-plants of rice. Scientica Sinica 17: 794-797

Tuvesson IKD, Pedersen S, Andersen SB (1989): Nuclear genes affecting albinism in wheat (Triticum aestivum L.) anther culture. Theor Appl Genet 78: 879-883

Xu ZH (1990): Barley (Hordeum vulgare L.): Anther culture and the production of haploids. In Y.P.S. (ed) Biotechnology in Agriculture and Forestry, Vol 12 Haploids in Crop Improvement I. Springer-Verlag Berlin Heidelberg, pp 125-175

TOWARDS ARTIFICIAL FERTILIZATION AND TRANSFORMATION IN PERENNIAL RYEGRASS

A.P.M. den Nijs, H.M. van der Maas, and
L.A.M. Hensgens

Centre for Plant Breeding Research, P.O.Box 16, Wageningen, the Netherlands, and
Department of Molecular Biology, Leiden University, the Netherlands

Summary

Grasses being recalcitrant crops in tissue culture, the application of biotechnological approaches to breeding research has been slow to develop. In the Netherlands a collaborative program was started several years ago, aiming at artificial fertilization via fusion of gametoplasts and genetic transformation via especially developed gramineous plant vectors and promoters. The program is executed by CPO at Wageningen and the MOLBAS group of Leiden University, and is financially supported by the Ministry of Agriculture's Programme committee on agricultural biotechnology PcLB.

The state of the art of this collaborative program is presented and possible applications to breeding are discussed. As an example, a few of the most damaging insect pests of temperate grasslands may be controlled by resistance brought about by genetic manipulation.

Introduction

Plant biotechnology has a growing influence on plant genetics and plant breeding research, and in some crops biotechnological approaches are already being incorporated into practical breeding programs.

Genetic manipulation including cell fusion and genetic transformation requires tissue culture techniques tailored to the crop species. Monocotyledonous crops such as small grains, maize and grasses belong to the more recalcitrant species in this respect. Perennial ryegrass is the major forage grass in NW Europe, and therefore we chose this species for cell biological and transformation research several years ago.

The last few years successes have been reported on the tissue culture of several important grass species. Among the temperate forage grasses, there are reports of regeneration from protoplasts of orchardgrass (*Dactylis glomerata*) and the ryegrasses (*Lolium perenne* and *L. multiflorum*) (review by Conger, this volume). Our joint research with the breeding company Barenbrug Holland on protoplast isolation, culture and regeneration of *Lolium perenne* yielded several green regenerated plants (Creemers-Molenaar, et al., 1988). Protocols are now being adapted to increase the efficiency of regeneration (Creemers-Molenaar and Loeffen, this volume).

The next step in a genetic manipulation approach is the genetic transformation of the target species with marker genes, and preferably genes for desired characters. Since grasses are not susceptible to the Agrobacterium-mediated gene transfer system, an efficient protoplast regeneration system is needed because of the usually low transformation frequencies obtained by direct gene transfer. Until now, transgenic grasses have only been obtained in orchardgrass (Horn et al., 1988). Unfortunately the few transgenic regenerants have proved sterile and weak (Conger, this volume).

The extremely low regeneration frequencies coupled with the low efficiency of direct gene transfer techniques led us to attempt a different approach which imitates natural fertilization at the cellular level. A molecular approach was added by bringing in expertise of the MOLBAS group at Leiden University, which specializes in designing gene constructs specifically for gramineous crops, mainly rice.

The program contains three elements, i.e gametoplast isolation and fusion, plant vector development and genetic transformation. Finally some possible applications are discussed.

Gametoplast isolation and fusion

Gametoplasts are protoplasts isolated from gametes. These protoplasts contain the haploid chromosome number and have no cell wall.

Fusion of gametoplasts derived from male and female gametes represents the ultimate stage of artificial fertilization. The fusion product can be considered as an artificial zygote or zygotic protoplast which may develop into an embryo because of its biological background. Alternatively a callus may develop which could regenerate somatic embryos. In this way a long callus phase is circumvented, which is often associated with somaclonal variation. Fusion of haploid gametoplasts also results in a diploid product, wherein specific interactions can be studied because both gametoplasts will contribute their nuclear genome as well as their cytoplasm including mitochondria and plastids. This contrasts with the process of natural fertilization with its limited transfer of cytoplasm through the male gamete.

In order to fuse "female" and "male" gametoplasts, one must first be able to isolate these protoplasts in reasonably large numbers. Further they must remain viable during the time needed for fusion experiments.

Sperm cell isolation

Preliminary studies showed that sperm cells of mature pollen of perennial ryegrass provide a good source of the "male" gametoplasts. An isolation procedure was developed (Van der Maas and Zaal, 1990).

The sperm cells are released from freshly collected pollen grains in a modified pollen germination medium by combination of mechanical pollen breakage and osmotic shock. After purification on a percoll gradient, the sperm cells are counted via phase contrast microscopy. Viability of the sperm cells is tested by staining with fluorescin diacetate.

Damage to the isolated sperm cells can occur due to oxidative stress and different enzymes like peroxidases which are released in the culture-medium due to the squashing. Optimization of the isolation procedure with several antioxidantia was therefore attempted.

The best treatment with a buffer containing fetal calf serum and Vitamin C, results in yields of 10-15% gametoplasts which remain viable for at least 48 hrs. During the following days the viability slowly declines until zero. The isolated sperm cells lack a cell wall, they appear to be true gametoplasts (Van der Maas et al., this volume). The characterization of the cells is focussed on the presence of mitochondria and proplastids.

Embryo sac and egg cell isolation

Egg cells are the source of the "female" gametoplasts. These egg cells usually lie well protected in an embryo sac covered by nucellus tissue and integuments together constituting the ovule. The isolation of the egg cells is a more arduous task both because of this protected site and the low numbers, since in the gramineae there is only one egg cell per flower.

For isolation of embryo sacs, usually ovules are dissected from ovaries from e.g. maize (Wagner et al., 1989). The ovules are macerated in an enzyme mixture of different enzymes involved in cell wall digestion. The embryo sacs are thereafter individually selected using a Pasteur pipette. Subsequently, isolation of the egg cell from the embryo sac is achieved by further digestion.

Before isolation from perennial ryegrass was attempted, the constitution of the cell wall of the embryo sac was studied. No callose and cutine were found, but deposition of cellulose was noticed in the wall of the embryo sac.

Several chemical and physical factors may affect the isolation. Among the former are the strength and composition of the enzyme mixture, the osmolality of the medium and pH. Physical factors include temperature, length of incubation and intensity of rotation. Experiments which vary the above factors are in progress, but the yield of embryo sacs is not yet sufficient to start fusions in the program (Van der Maas et al., 1990)

One-to-one fusions of "female" and "male" gametoplasts have very recently been performed in maize (Kranz et al., 1990). The isolated egg cells did not divide in culture, but grew into multicellular structures only after fusion with a sperm cell derived gametoplast. This is the first example of an artificial zygote in the plant kingdom.

Plant vector development

Transformation requires plant vectors with selection and/or reporter genes, present in the plasmid DNA used for the direct gene transfer.

Although several standard chimaeric gene contructs are available, there is evidence that in gramineous plants the expression of plant vectors developed for dicotyledonous plants is lower.

Therefore specific plant vectors are being designed for some important gramineous crops such as rice and perennial ryegrass by the MOLBAS-group at Leiden University. Chimaeric plant vectors were constructed, which contain a defined gene for resistance to the antibiotic hygromycin as a selection marker. This gene is under control of the 35 S CAMV promoter.In addition the vectors contain the GUS gene as a reporter gene which can be visualized by adding the substrate X-gluc yielding a blue precipitate in situ. The GUS gene is under control of a T-DNA gene for mannopine from Agrobacterium. With these vectors several dicotyledonous species and rice have been stably transformed (Hensgens et al., 1990).

For the practical application of genetic manipulation of perennial ryegrass, tissue specific and constitutive promoters must be available, which are active in the intact plant. To obtain such promoters for rice, a cDNA bank was constructed using mRNA from Oryza Indica IR36 plantlets. From this gene bank cDNA clones were selected via differential hybridization which correspond to mRNAs that are specifically and strongly expressed in roots or in shoots or in all plant tissues.

The genomic counterparts of the cDNA clones were isolated to obtain promoter regions. With these genes promoter and translational fusions with the GUS gene have been constructed (Hensgens et al., 1990). The chimaeric genes will be used in transformation experiments in perennial ryegrass, to find out whether these promoters can indeed direct tissue specific or constitutive expression.

It is not certain that rice promoters will work in Lolium as they do in rice. To establish conserved regions essential for expression, Lolium genes which are homologous to the three characterized rice genes were isolated. Screening of a genomic Lolium bank yielded several clones for two of the rice cDNA's, and finally one for the root specific gene (Hensgens, pers. comm.).

After comparison of the promoter sequences with those of rice, promoter fusions will be made with the reporter genes and the expression of these vectors will be studied in transformation experiments of rice and perennial ryegrass.

Genetic transformation

Parallel with the gametoplast isolation, the conditions for genetic transformation are examined. A transformation procedure is developed to test the isolated promoter sequences from rice and perennial ryegrass. Protoplasts from fast growing cell suspension cultures are used (Creemers-Molenaar et al., 1988).

The transformation method is a direct gene transfer procedure based on incubation of protoplasts and the DNA in the presence of polyethyleneglycol (PEG) followed by incubation in a medium with a high salt concentration (Krens et al., 1982). At 13.6% PEG various parameters for transformation were tested. Under the selection criteria used, some calli were selected and are now investigated at the DNA level.

Possible applications

The above described program aims to make available to perennial ryegrass breeder novel methods to introduce genetic variation by gametoplast fusion and genetic transformation. For the latter technique genes for agronomically desirable characters are needed. We must realize that the vast majority of such characters has a too complex (genetic) basis for this type of manipulation. In fact, they are already difficult to manage in the conventional breeding process. What agronomically desirable characters might then be accessible to molecular breeding?

Tolerance to broad spectrum herbicides is desirable for maintaining a clean crop both in seed production and in the meadow, but the strategy to minimize environmental hazards in grassland management makes this trait less desirable.

A number of viruses include the ryegrasses among their hosts. Estimates for yield losses due to barley yellow dwarf virus in perennial ryegrass run up to 20%. In some varieties levels of tolerance are present. The introduction of resistance e.g. based on the coat protein gene of this virus appears to be attainable and attractive.

However, the most important pests of temperate grasslands are insects. Leather-jackets (larvae of Tipula paludosa), cutworms (Agrotis spp.) and grass grubs (e.g. Melolontha melolontha) cause heavy damage to pastures and a significant amount of general purpose insecticides is sprayed to combat these pests. Other ways of managing these pests are therefore both economically and environmentally attractive. In Wageningen and elsewhere genes are being identified for toxic crystalline proteins of Bacillus thuringiensis (Bt), which have insecticidal properties to specific plague insects. The larvae of Tipula paludosa are among the target species for biological control methods involving Bt-gene transformed rhizosphere bacteria, but these require very careful management (Waalwijk, 1990).

The relevant Bt-toxin gene could also be transferred directly into the grass plant. When other genes become accessible with general insecticidal properties such as proteinase-inhibitors, these could also be tested for their effect on the above pests.

Bacteria and fungi threaten grass production and palatability depending on the region. Among these Xanthomonas appears to gain importance. Molecular breeding might be useful to introduce general resistance to this class of pathogens by introducing genes for thionins. Thionins are small peptides present in several cereals which show anti-bacterial and possibly anti-fungal activity. Synthetic genes for these thionins have been constructed by CPO (Stiekema, pers. comm.).

Forage quality may also be a future target for genetic manipulation. At least under the present dutch conditions, the nitrogen level of the forage is much too high for optimal conversion by the bacterial flora in the fore stomach of ruminant cattle. Mixed feeding with e.g. corn silage but also large amounts of concentrates has become popular with farmers to reduce the nitrogen dosage and increase energy input.

A lower nitrogen level in the crop due to lower fertilizer applications leads to less yield, and we have found little genetic variation for N-efficiency. However, the distribution of nitrogen over parts of the grass plant may be influenced by manipulating key enzymes in the nitrogen metabolism. Genetic transformation with known genes from other species may be a valuable approach, but first detailed studies of the nitrogen distribution are necessary.

Another very desirable character for a seed sown crop like perennial ryegrass is a certain way to capitalize on heterotic and epistatic gene interactions by apomixis. Apomixis occurs in several grasses, e.g. in Kentucky bluegrass and some tropical forage grasses. Apomixis is a powerful means to transmit elite gene complexes, but it generally hampers breeding efforts unless it can be experimentally modified. The genetic base of most known forms of apomixis is rather complex, but the sensitivity of the apomictic process to growth hormones has stirred interest in isolating genes involved in apomixis. If such genes can be transferred into perennial ryegrass, apomixis could become an asset to the breeder.

The above potential benefits from biotechnology require genes to be transferred and expressed. The protoplast fusion technique can be applied without this prerequisite. Thus wide crosses such as the combinations yielding the diverse types of Festulolium can be repeated by protoplast fusion, and the stability and phenotypes of these novel hybrids can be studied. A more realistic alternative to this might be asymmetric fusion, whereby limited genomic and/or organelle DNA of one partner can be combined with the genome/plasmon of the other species. This technique could render cytoplasmic male sterility much easier to handle, and thus open up possibilities for hybrid breeding in perennial ryegrass.
However, we fully realize that these applications will not be available in the near future, and much more basic research is needed.

Acknowledgement

The joint program in grass cellular and molecular genetics is being financially supported by the Programme Committee on Agricultural Biotechnology PcLB of the Dutch Ministry of Agriculture, Nature Management and Fisheries.

References

Creemers-Molenaar, T., Loeffen J.P., Zaal M.A.C.M., 1988. In: K.J. Puite, et al. (eds): Proceedings of the 7th International Protoplast Symposium, Wageningen, 1987. Kluwer Academic Publishers, Dordrecht, Boston, Londen, p. 53-54.

Hensgens, L.A.M. and P.E. van Os-Ruygrok, 1990. Design of gene constructs for transformation in grasses. In: Dekkers, J.J. et. al. (eds), Agricultural biotechnology in focus in the Netherlands, PUDOC, pp. 25-30.

Horn, M.E., R.D. Shillito, B.V. Conger & C.T. Harms, 1988. Transgenic plants of orchard-grass (Dactylis glomerata L.) from protoplasts. Plant Cell Reports 7: 469-472.

Kranz, E. J. Bautor and H. Loerz, 1990. In vitro fertilization of single isolated gametes of maize mediated by electro-fusion. Sex. Plant Repr. in press.

Krens, F.A., Molendijk, L., Wullems, G.J., Schilperoort, R.A., 1982. In vitro transformation of plant protoplasts with Ti-plasmid DNA. Nature 296: 72-74.

Maas, H.M. van der, Zaal, M.A.C.M. Sperm cell isolation from pollen of perennial ryegrass (Lolium perenne L.) In: B. Barnabás and K. Liszt (eds): Proceedings of the third meeting of The Sperm Cell Club, Martonvásár, 1989.

Maas, H.M. van der, F.A. Krens, M.C.A.M. Zaal en H.J. Huizing, 1990. Gametoplast fusions and DNA-transfer in grasses. In: Dekkers, J.J. et al. (eds). Agricultural biotechnology in focus in the Netherlands, PUDOC, pp. 21-25.

Waalwijk, C., 1990. General Introduction to biotechnology in crop protection. In: Dekkers, J.J. et al. (eds.) Agricultural biotechnology in focus in the Netherlands. pp. 51-52.

Wagner, V.T., Song, Y.C., Matthys-Rochon, E., Dumas, C., 1989. Observations on the isolated embryo sac of Zea mays L.. Plant Science, 59: 127-132.

GENOME ANALYSIS AND ITS MANIPULATION IN LOLIUM

G M Evans[1], M D Hayward[2], J W Forster[1], N J McAdam[2], M J Scanlon[1], M Stammers[1] and J A K Will[1].

[1]Department of Agricultural Sciences, University College of Wales, Aberystwyth, U.K. and [2]AFRC Institute for Grassland and Environmental Research, Welsh Plant Breeding Station, Aberystwyth, U.K.

Summary

The availability of a comprehensive genetic map of any crop is of considerable value both in basic research and in the production of improved cultivars through marker linked selection. This paper describes the initial stages of producing such a map of the Lolium genome using a combination of morpho-physiological, isoenzyme and RFLP markers.

Heterologous cDNA together with homologous cDNA and genomic probes are being assessed for their potential in detecting RFLP polymorphism. Classical genetic analysis using progeny derived by several crossing schemes is being used to construct a linkage map of the different marker loci and these are allocated to specific chromosomes by means of trisomic analysis. The possible uses of such a map are listed.
Keywords: Lolium, genetic map, RFLP, isoenzyme.

Introduction

The idea of marker linked selection is not new. It has long been argued that it should be possible to simplify the production of new cultivars by selection of marker genes which are known to be linked to loci controlling important agronomic or physiological characteristics. Such a scheme demands a detailed genetic map of the crop species being manipulated. Until recently, however, our capacity to achieve this was constrained by the lack of a significant number of marker genes. A limited range of morphological and physiological markers has been available for many crops supplemented during the last twenty years by an array of isoenzyme loci. Nevertheless, genetic analysis has still been restricted to whole genome analysis or at best to single chromosomes or chromosome arms. More recently, however, the development of techniques for identifying RFLP loci has had considerable impact on genetic mapping in many crop species. Reasonably detailed genetic maps have already been reported for several crops (Tanksley et al., 1989; Gebhardt et al., 1989; Chao et al., 1989; Slocum et al., 1990). This present paper is a preliminary report of our programme to produce such a genetic map of the genus Lolium.

The basic requirements for creating a genetic map of "marker" loci can be summarized as follows: (1) sexual reproduction; (2) selectively neutral markers; (3) genotypes which are polymorphic at as many loci as possible; (4) the capacity to classify a meaningful number of segregant progeny and (5) a system to allocate linkage groups to individual chromosomes.

Breeding system in Lolium

The genus Lolium is made up of only six species all of which reproduce sexually. Although three out of the six are self-pollinating the important agricultural species are the outbreeding *L. perenne* and *L. multiflorum*. Individuals within these two species are, naturally, heterozygous and consequently are equivalent to F1s in breeding terms and can therefore be used directly for mapping purposes. The inbreeding species such as *L. temulentum* are, nevertheless, useful as testers on account of their homozygous nature.

Marker loci in *Lolium*

The initial stages of any mapping exercise is always constrained by a lack of suitable markers. Our programme in *Lolium* is no exception and the utility of morpho-physiological, isoenzyme and RFLP loci is being examined for this purpose. Recent developments involving random amplification of polymorphic DNA (RAPD) could possibly add a fourth source of useful variants.

A limited collection of morpho-physiological mutants has been assembled in various genotypes of several species of *Lolium*. The early work of Jenkin (1928) showed independent inheritance for some of the morphological markers available at that time. Apart from their paucity and occasional deleterious effect on vigour, the dominant/recessive nature of gene action at these loci restricts the type of linkage analysis which is appropriate.

On the other hand the codominant nature of both isoenzyme and RFLPs allows complete classification of all segregants. Moreover, the existence of multiple alleles at many isoenzyme, and we suspect, RFLP loci increases the chances of identifying suitable polymorphisms. To date 20 polymorphic isoenzyme loci have been identified in *Lolium*. Although this number of marker loci represents an impressive improvement on that available previously, it is still inadequate for detailed mapping of the genome.

In a series of experiments using both isoenzyme and morphological markers a number of linkage groups have now been established in *L. perenne*. Whilst some of the data requires larger samples and further families to be analysed three main linkage groups have been identified. These are shown in Figure 1 from which it can also be seen that two have also been allocated to specific chromosomes.

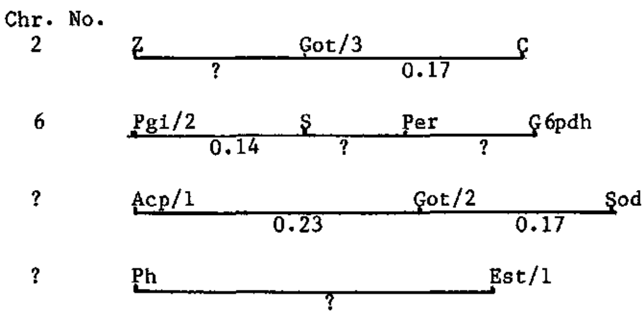


Fig.1 Linkage groups in *L. perenne*.

RFLPs appear to offer a much better opportunity of obtaining markers at close intervals. Single or low copy DNA is extracted and cloned from the species being mapped or from a related species and then used as a probe to follow the segregation of homologous "loci" in segregants from a suitable breeding programme. Tanksley et al. (1989) have summarised the important comparisons between RFLP and morphological markers. Their main characteristics resemble those of isoenzyme markers but with the all important difference that the number available in a single species appears to be limitless.

RFLP probes

We have used DNA probes from a number of sources in order to detect Restriction Fragment Length Polymorphism in the *Lolium* genus.

(1) Heterologous probes

A number of uncharacterised cDNAs from maize (*Zea mays*) which had been used in maize mapping and defined as low or single copy sequences (Helentjaris, pers. comm.) were used initially. Although a some of these probes showed homology to *Lolium* DNA, we have since preferred to use cDNAs of defined function obtained from members of the Gramineae which are more closely related to the ryegrasses, such as wheat and barley. These include wheat E_m (early methionine labelled peptide), *rbcS* (small subunit of ribulose bisphosphate carboxylase-oxygenase), *cab* (chlorophyll a/b binding protein), *amy1* and *amy2* (α-amylase) cDNAs, and the barley β-amylase and *nir* (nitrate reductase) cDNAs. All these have proved useful clones, although some of them correspond to small gene families. The complexity of hybridisation patterns to *Lolium* DNA is compatible with similar copy number per haploid genome as in the species of origin.

(2) Homologous probes

(a) cDNA clones have been derived by random primed cDNA synthesis on mRNA purified from the coleoptile of *L. multiflorum*. Non-green tissue was chosen in order to reduce the prevalence of photosynthetic (*rbcs*, *cab*) cDNAs in the library. The cDNA was blunt end cloned into the *SmaI* site of pUC18. The resulting library (c. 200 clones) was characterised by plasmid digestion, revealed an insert size range from 0.5 - 5kb. The clones have been probed with labelled total RNA and total DNA in order to assess both the level of expression of the corresponding genes and their copy number. A number of apparently high copy clones have been identified, presumably corresponding to contaminating structural RNAs. We are concentrating on low copy, low abundance cDNAs in order to maximise the diversity of the selected group.

(b) genomic clones have been obtained by cloning of size fractions from restriction enzyme digests into plasmid vectors. Both non-methylation sensitive (*BamHI*) and methylation sensitive (*PstI*) enzymes have been used. Screening of the clones with labelled total DNA has indicated that as for maize and tomato, although the *BamHI* library contains a preponderance of repetitive clones (c. 75%), the *PstI* library (c. 200 members) is highly enriched for low copy sequences.

We have also recently begun to explore the application of the RAPD method (A. Rafalski, pers. comm.) to molecular marker studies in the forage grasses. This technique relies on the use of random 10-mer primers to amplify a discrete set of dispersed 'loci' from total DNA by the polymerase chain reaction. Although we have experienced some problems with reproducibility, the use of two such primers on a set of

L. perenne genotypes demonstrates that the RAPD method produces a number of bands in a reasonable agreement with theoretical predictions. Some bands are invariant whilst others produce a genotype specific pattern. Parallel studies on a L. perenne x L. temulentum family (details below) have shown the presence of heterozygous RAPD loci in L. perenne.

Breeding Strategy

Segregating progeny are being generated from several breeding schemes involving three of the six species of Lolium. In this way we are able to utilise existing programmes. The initial mapping of isoenzyme loci by Hayward & McAdam (1989) was performed on the progeny of a cross between two heterozygous individuals. This, in genetic terms, is equivalent to an F_2. Lawrence, Cornish & Hayward (1979) drew attention to the benefits resulting from the codominant nature of the multiplicity of isoenzyme alleles in allowing complete classification of the progeny from such a cross. The same attributes apply to RFLPs.

The codominant nature of both types of markers also simplifies the use of heterozygous x homozygous crosses. Any such cross becomes equivalent to a backcross to a homozygous recessive as used in classical three point linkage analysis of morphological markers. A L. perenne genotype (E5/2/5-10) derived from a complex three way cross is now being used in a programme of this type. Progeny of a test cross to the homozygous L. temulentum line Ba 3081 is being used for mapping. The use of a self-pollinating L.temulentum as a tester should avoid problems associated with linkage to the self-incompatibility system in Lolium.

A slight modification on this scheme has also been used to generate suitable progeny. This involves crossing a L. perenne x L. multiflorum F_1 to a doubled haploid L. perenne tester.

A different strategy is to directly sample the products of meiosis from a heterozygous plant through the use of androgenetic haploids obtained by anther culture. The haploids themselves or their dihaploid derivatives are then assayed for isoenzyme and RFLP markers. Recombination values between such loci should be identical to those obtained by the testcross method described above. However, distorted segregation ratios have been recorded at some isoenzyme loci in dihaploid families of L. perenne thus limiting their utility (Hayward et al., 1990).

RFLP polymorphism

Assessment of the degree of polymorphism in L. perenne is being done with heterologous probes, mostly from wheat, and the homologous cDNA and genomic clones. Seven diverse genotypes comprising heterozygous outbred genotypes, androgenetic dihaploids derived from one of them and an F_1 hybrid are used as an initial screen. The low/single copy heterologous probes show varying results depending on the enzyme used and the copy number (related to the number of bands) of the gene or gene family. For example, the wheat α-amylase 1 cDNA showed c.10 bands in EcoRI digests, two of which were present throughout the seven genotypes. None of the 12 bands present in DraI digestions were present in all genotypes.

When used as a probe against the E5/2/5-10 x Ba 3081 family, most of the bands are invariant. A comparison of progeny from this cross showed one or two segregating bands. We have demonstrated a similar result in this family with the other heterologous probes rbcs, E_m, β-amylase and cab; for example, the rbcs probe hybridises to eight major bands in the progeny, four bands derived from L. temulentum, three monomorphic bands from L. perenne and one pair of allelic bands 16 and 5.4 kb. Hence these heterologous probes are immediately available for mapping this family.

The homologous cDNAs and genomic clones show in general much simpler patterns than the heterologous cDNAs. RFLP has been detected in both the screening genotypes and the interspecific cross family. Clones which have been identified as indicators of heterozygosity in this family will be used against panels of 50+ progeny to obtain linkage data

Chromosome Assay

The allocation of linkage groups to individual chromosomes, although not essential, is nevertheless a convenient way of representing the location of markers. The conventional method of achieving this is through the use of heterozygous aneuploid stocks. Monosomic lines present the best option but these are rare in diploids although they do exist in maize (Helentjaris, Weber & Wright, 1986). Alternatively, trisomics can be utilised but these require a more elaborate analytical procedure. The use of trisomics to assign the Got/3 and Pgi/2 genes to specific chromosomes of L. perenne has already been described by Lewis, Humphreys & Caton (1980). Trisomics in a perennial grass such as L. perenne can of course be propagated clonally and are therefore easy to maintain. However, it is implicit from their breeding system that each trisomic will be genetically different and this must limit their value to some extent. Moreover, by the same reasoning, they would be unsuitable for further breeding to produce telotrisomics. A sexual generation whether by selfing or outcrossing would increase the hetero-geneity of the material even further. We therefore considered it desirable that a complete trisomic series should also be constructed in the self pollinating L. temulentum with a completely homozygous line (Ba 3081) of known pedigree used for this purpose.

Colchicine induced autotetraploid plants were crossed to the original diploid homozygous line and the resulting triploids backcrossed once more to the same diploid line. A high proportion of the progeny of such a cross are trisomic. The chromosome status of the final progeny is ascertained by chromosome counting of root meristem cells at c-mitosis and by examination of first metaphase of meiosis. Identification of the seven different trisomics can be achieved partially on the basis of chromosome morphology although complete separation of the smaller ones is particularly difficult. The final classification will be on the basis of genotype.

The allocation of linkage groups to specific trisomics can be achieved in two ways. Both involve, in the first place, crossing the homozygous trisomics of known genotype with stocks of different marker genotypes. One method involves classifying the progeny directly for the markers being mapped using increased density of isoenzyme stain or of probes in RFLP Southern blots to detect double and single alleles in the test cross progeny. The presence of two doses of one allele and one of the other in a single progeny plant would indicate a trisomic and that the locus being mapped is on that particular trisomic. The chromosome status can be checked, if necessary, by examination of c-mitosis in the root meristem.

Alternatively, trisomic progeny from the first test cross can be identified cytologically and then crossed to a second tester carrying a third allele of the marker. The presence of a triple heterozygote phenotype in the progeny would indicate the location of the marker on that trisomic.

Future refinement of in-situ hybridization techniques could result in a cytological method of chromosome mapping. Single or low copy DNA probes developed for RFLP mapping could be labelled with a suitable detection system and used for hybridizing directly on to chromosome preparations of trisomics. Visual location of the hybridized probe would indicate the position of the RFLP loci.

Prospects

The utility of any marker system is based on identifying close linkage between the markers and other genes of importance. The incorporation of such a system into aspects of genetic analysis and breeding has already been reported for several crops and its potential discussed in detail by Hayward (1987) and Tanksley et al. (1989). Briefly, this would include (1) the measurement of variability in populations, (2) the analysis of Quantitative Trait Loci (QTL), (3) monitoring the segregation of important characteristics in different types of breeding programmes, (4) identification of "alien" genetic material in interspecific and intergeneric hybridisation programmes, (5) cultivar discrimination in outbreeders and determination of varietal purity in hybrids and inbreeders. and (6) establishment of evolutionary relationships between species and genera.

References

Chao, S., P.J. Sharp, A.J. Worland, E.J. Wareham, R.M.D. Koebner & M. D. Gale, 1989. RFLP based genetic maps of wheat homoeologous group 7 chromosomes. Theor. Appl. Genet. 78: 495-504.

Gebhardt, E., E. Ritter, T. Debener, U. Schachtschabel, B. Walkemeier, H. Urig & F. Salamini, 1989. RFLP analysis and linkage mapping in Solanum tuberosum. Theor. Appl. Genet. 78: 65-75.

Hayward, M.D., 1987. Isoenzymes in plant breeding. J. Ag. Sci. U.C.W. Aberystwyth 67: 177-215.

Hayward, M.D. & N.J McAdam, 1989. Linkage relationships in Lolium. IGAP Welsh Plant Breeding Station Ann. Report for 1988: 30.

Hayward, M.D., A. Olsen, I.K. Due, R. Jenkins & P. Morris, 1990. Segregation of isoenzyme marker loci amongst androgenetic plants of Lolium perenne. Plant Breeding 104:68-71.

Helentjaris, T., D.F. Weber & S. Wright, 1986. Use of monosomics to map cloned DNA fragments in maize. Proc. Natl. Acad. Sci. USA 83: 6035-6039.

Jenkin, T.J., 1928. Inheritance in L. perenne L. I. Seedling characters, lethal and yellow tip albinos. J. of Genet. 19: 391-402.

Lawrence, M.J., M.A. Cornish & M.D. Hayward, 1979. Simultaneous estimation of male and female linkage from a single intercross family. Heredity 43: 107-121.

Lewis, E.J., M.W. Humphreys & M.P. Caton, 1980. Chromosome location of two isozyme loci in Lolium perenne using primary trisomics. Theor. Appl. Genet. 57: 237-239.

Slocum, M.K., S.S. Figdore, W.C. Kennard, J.Y. Suzuki & T.C. Osborne, 1990. Linkage arrangement of restriction fragment length polymorphism loci in Brassica oleracea. Theor. Appl. Genet. 80: 57-64.

Tanksley, S.D., N.D. Young, A.H. Peterson & M.W. Bonierbale, 1989. RFLP mapping in plant breeding: New tools for an old science. BioTechnology 7: 257-264.

BIOTECHNOLOGY IN FORAGE GRASS BREEDING

B. V. Conger

Department of Plant and Soil Science, University of Tennessee, Knoxville, TN 37901-1071 USA

Summary

Research progress in plant biotechnology has been rapid in recent years. However, the practical applications have been slow to develop. This is especially true for gene transfer where no improved plants or plant products have yet reached the marketplace. Most of the research focused on practical applications is being conducted by commercial firms and therefore, emphasis is on major crop plants such as maize, soybean, cotton, wheat, etc. Genetically engineered plants for herbicide, disease and insect tolerance are expected to be commercially available within the next 5 years. The state-of-the art of both basic and applied research with forage grasses lags far behind that of major cash crops. Regulations concerning testing and release of genetically transformed organisms must be addressed and followed. Approval for release of engineered forage grasses may be more difficult to obtain than for other crops because of their less domestication and closer relationship to weed species.
Keywords: Gramineae, genetic engineering, tissue culture.

Introduction

Progress in plant genetic research has been both rapid and remarkable during the past decade. It is now possible to isolate, clone and sequence genes. It is also possible to insert them into other species and have them expressed. It is even possible to transfer bacterial, insect and animal genes to higher plants and have them expressed.

Despite this rapid progress, there are still no genetically engineered plants or plant products in commercial use. However, extensive field tests are underway and, genetically engineered crops for viral, insect and herbicide resistance should be in the marketplace by the mid 1990's.[39]

Most of the work directed toward application of this technology is being conducted by commercial firms. Currently, there are about 500 companies worldwide having at least some interest or activity in agricultural biotechnology.[46] Because of the extensive involvement and investment by commercial firms, the concentration has been on major cash crops such as maize (Zea Mays) and soybean (Glycine max). Individual forage grass species are receiving much less attention and financial support. Therefore, the state of technology with these species lags far behind. This presentation will focus on various applications of plant biotechnology, including gene transfer, and describe the current status with forage grasses.

Anther Culture and Haploids

The production of haploids with ease and in great numbers has been of long interest to grass breeders. Such plants might be used to develop homozygous breeding lines, which in many forage grasses is very difficult because of self-incompatibility, natural outcrossing and inbreeding depression. Bingham[3] has conducted both practical and theoretical research with tetraploid derived diploids to maximize heterozygosity in alfalfa

(Medicago sativa). Such schemes might be applicable to certain polyploid grass species if plants possessing the gametic chromosome number could be readily produced.

Anther culture technology and production of haploids is much more advanced for the major cereals than for forage grasses. In fact, new cultivars have been developed and released using anther culture techniques. A notable example is 'Florin' wheat (Triticum aestivum).[8] An apparent breakthrough resulted with the recovery of haploid tall fescue (Festuca arundinacea) plants from anther-panicle cultures.[25] However, the technique has not been repeated in either tall fescue or any other forage grass species. Furthermore, and unfortunately, there has been little progress since that time in obtaining haploids in forage grasses. Only a few papers have been published in the past 10 years on anther or pollen culture experiments with these species compared to the few hundred for the various cereals. There have been apparently few or no attempts to produce haploids via the Hordeum bulbosum[24] or related procedures which have been quite successful in cereals. Recent reports describe obtaining haploids in wheat[29] and oats (Avena sativa)[37] after crosses with maize.

This area is one of high potential for forage grass improvement. Given sufficient resources, success should be possible. There is need for a concentrated effort utilizing the already obtained and reported knowledge from research with cereals.

Clonal Propagation

The advantages of vegetative propagation of outstanding phenotypes in forage grasses are well known and the successful exploitation of it is especially exemplified in the bermudagrass (Cynodon dactylon) improvement program at Tifton, Georgia. However, many important forage grasses, e.g., cool season species with a bunch type growth habit, do not possess plant parts, such as rhizomes or stolons which can be readily used for vegetative propagation. Therefore, micropropagation through cell and tissue culture techniques or so-called synthetic seeds is potentially attractive to grass breeders.

Micropropagation depends on efficient and repeatable regeneration systems. The most logical propagules are somatic embryos. In most grass species, regeneration systems are not highly enough developed to consider such use. A potential system exists with orchardgrass (Dactylis glomerata). More than 2,000 somatic embryos can be produced in 30 ml of liquid medium[16] and they will develop to a germinable stage.[17]. However, certain refinements are needed for a functional synthetic seed system. One is synchronization of embryo development. Within a culture flask, developmental stages range from young proembryo to fully mature. Abscisic acid (ABA) effectively regulated and synchronized embryogenesis in suspension cultures of caraway (Carum carvi)[2] but its effectiveness has not been demonstrated in other species. In orchardgrass, ABA decreased precocious germination; but it also greatly decreased embryo number.[6]

Other requirements might include induction of quiescence or embryo dormancy and encapsulation of embryos. Orchardgrass somatic embryos were dehydrated to 13% water.[18] After a 21 day storage period, 4% germinated and produced plants. Obviously, this must be greatly improved for practical use of the system. Embryo quality and developmental stage would determine germinability. Depending on the method of sowing, encapsulation may or may not be needed. A method of field establishment such as the bandoleer system described by Hauser[20] may not require encapsulation since the seedlings are established and then transplanted with a special machine.

Encapsulation physically protects the somatic embryo and can provide nutrients, growth regulators, fungicides, etc.[19] Both wet and dry encapsulations have been considered. A problem has been low conversion rates from artificial seed to plant. One of the better systems is with alfalfa in which somatic embryos are encapsulated in alginate. The recovery rate was 60% under in vitro conditions but only 20% under non-sterile soil conditions.[13]

Probably the most serious limiting factor is cost. Not only is much investment still required for development and refinement of the technology but also of great importance is the cost per propagule for a particular crop. The cost for producing 14-day-old greenhouse transplants from alfalfa somatic embryos is 3.3 cents per unit whereas that for true seeds is 0.0008 cents per unit.[34] Undoubtedly, this cost difference is far too great for agronomic crops and especially for forage grasses where the value per unit area is relatively low. Initial application of this technology is most likely to be with high value horticultural or tree crops.

Somaclonal Variation

That genetic and chromosomal changes are induced by cycles of dedifferentiation and redifferentiation in plant cell and tissue cultures is well known. This phenomenon has been termed somaclonal variation.[28] During the past decade, numerous examples have been published for numerous crops.

Ahloowalia[1] was among the first to report on forage grasses. He found both chromosome loss and structural changes in Lolium multiflorum and L. perenne plants regenerated from tissue cultures. We observed both phenotypic variation and chromosomal abnormalities in tall fescue regenerants.[35] Lagging chromosomes and micronuclei in microspores were the most common aberrations but 2 trisomics and 1 monosomic were also found among the 53 plants studied. None were found among 21 controls. In a more recent study with tall fescue, 59 of 166 somaclones, were aneuploid ($2n = 38, 39, 40$ and 41) and 8 were of higher ploidy ($2n = 56, 70, 78$ and 80).[9] The production of aneuploid or polyploid series in open pollinated forage grasses may be one of the best uses of somaclonal variation in these species.

Somaclonal variation may also be a useful tool to generate variability in apomictic plants. Taliaferro et al.[42] observed morphological variation and changes in chromosome number, including aneuploids, among R_1 plants of old-world bluestem grasses (Bothriochloa sp.). These authors further suggest a potential of developing aneuploid stocks that can be correlated with reproductive modes, thus permitting determination of the chromosomes carrying genes for apomixis.

Wide Hybridization

Two areas involving in vitro culture for wide crosses deserve mention. These are protoplast fusion (parasexual hybridization) and embryo rescue.

1. Protoplast Fusion

One of the most significant events for stimulating interest in cellular approaches to plant improvement was the demonstration of parasexual interspecific plant hybridization by Carlson et al.[5] Parasexual hybrididization has been of little value, thus far, in gramineous species. However, its use to transfer male sterility from indica to japonica rice

(Oryza sativa) indicates a potential application of great value.[27] Somatic hybridization of rice and barnyardgrass (Echinochloa oryzicola) was reported by Terada et al.[44] However, most regenerated shoots were abnormal and the few resultant plantlets, although clearly different in morphology from either parent, could not be established in soil.

Parasexual hybridization is not likely to make a significant contribution to forage grass improvement in the near future. The technology has been of limited practical value for any crop species and the difficulties with protoplast regeneration from grass cell cultures is a severe impediment. If these difficulties could be overcome, one could envision highly useful interspecific or intergeneric hybrids, e.g., between Festuca and Lolium where conventional procedures have already produced hybrids between some species.

2. In Vitro Hybridization and Embryo Rescue

These techniques have been used successfully to make hybrids in several crop species.[47] However, with the exception of maize, there are no reports of successful crosses in the Gramineae or even of successful self-pollinations of ovules in vitro.[47] The use of embryo rescue and recovery of a hybrid plant (sterile) between Festuca arunidnacea and Dactylis glomerata[30] has not been repeated.

Conventional procedures have been used to make numerous interspecific and even intergeneric crosses between forage grass species and, at the present time, these would seem to be preferable to elaborate procedures involving in vitro culture.

Gene Transfer

Reports of genetic transformation in higher plants were first published in 1984.[7,23] Since that time progress has been remarkable and more than 30 plant species, including some major crops, have been genetically engineered.[14] There are currently numerous field tests, worldwide, with transgenic plants and products may be in the marketplace within 5 years. The first crops are likely to be solonaceous species, e.g. potato (Solanum tuberosum) and tomato (Lycopersicon esculentum genetically engineered for herbicide, virus or insect tolerance.[11,14,45]

As with most other areas of plant biotechnology, progress has lagged behind in important crops of the grass family. A primary reason is that early gene transfer experiments relied on the Agrobacterium vector system which works very efficiently in dicotyledonous species but does not normally infect gramineous species.

Until recently, the only method that yielded transgenic cereal plants was direct uptake of DNA by suspension culture derived protoplasts and then whole plant regeneration. Progress has been slow because plant establishment from protoplasts in a cereal species is very difficult and was not accomplished until 1986 when it was reported with rice (Oryza sativa) by three different groups (see recent reviews).[6,31] The first fertile transgenic cereal plants also were rice from transformed protoplasts.[40] The first genetically engineered maize plants obtained by this method were both male and female sterile and morphologically stunted.[36] The recent recovery of fertile transgenic maize plants represents a major advancement.[12,15] In these experiments, embryogenic suspension cells were transformed after bombardment with DNA-coated microprojectiles. This technology has generated much current interest as a DNA delivery system. It appears to hold special promise for transforming gramineous species because it requires neither the use of Agrobacterium nor regeneration from protoplasts. Efficient and repeatable regeneration from tissues and cells, especially across a wide range of species and genotypes, remains a major impediment to genetic engineering of cereals and grasses. The only example of genetic transformation among forage grasses is with orchardgrass.[22] This work utilized protoplasts isolated from embryogenic suspension cultures, direct uptake of a DNA plasmid containing a hygromycin resistance gene and whole plant regeneration. However, the plants could not be induced to flower and the one which we had in our laboratory lacked severely in vigor and finally died. Both the leaf and suspension culture systems in this species offer attractive possibilities for gene transfer experiments with microprojectiles. Preliminary efforts were unsuccessful (unpublished data) but new attempts are being initiated.

RFLPs and DNA Fingerprinting

There is much current interest in the development and use of restriction fragment length polymorphisms (RFLPs) to relate genetic maps, and even quantitative traits, to restriction fragments.[32,33,43] Again, much of this work is being conducted by commercial firms and therefore the major cash crops mentioned above are receiving the most attention. Because of the lower economic interest, and the lack of good marker genes, the technology will develop much slower for forage grasses. One potential use of restriction fragment mapping for grasses, however, is that of "fingerprinting" for cultivar identification and protection, especially in vegetatively propagated species. Such a program has been initiated with centipedegrass (Eremochloa ophiuroides) at The University of Tennessee (L. M. Callahan, personal communication).

Regulations, Field Testing and Release

There is much concern about how an engineered organism will interact with the particular environment into which it will be introduced. Keeler[26] asks "What is a hazardous genetically engineered organism?" She states that we really do not know; however, for such an organism to create problems when released into the environment all of the following are required: (1) possess a gene for a hazardous trait, (2) survival in the environment, (3) multiplication of the organism, (4) contact with species or biological systems that can be injured and (5) cause harm.

Genetically engineered crop plants are considered safe.[26] They are not generally a health hazard to people or animals; they are raised under close supervision; many are harvested at the end of the season and plants are not very mobile. Also, plants have been crossed (traditional genetic engineering) by man for centuries.[4] Brill[4] also points out that new variants resulting from conventional breeding have not caused serious problems. Most of our high yielding crops, productive forest trees, popular ornamentals and garden plants have been derived through breeding programs. Some crosses include those that would not have occurred without man's intervention and between species which do not readily cross pollinate. Furthermore, protoplast fusions and induced mutations have resulted in new variants which have caused no problems.

So, where is the problem? Critics argue that, traditionally, most of the traits plant breeders have introduced into crops, such as dwarfing, absence of dormancy, nonshattering and uniform maturity are traits that would have an adaptive disadvantage in the wild.[10,21] In contrast, many of the traits genetic engineers are interested in transferring might confer an adaptive advantage over a wild plant. These include, tolerances to drought, insects, diseases, salinity, frost and herbicides. Also, as pointed out by Hoffman,[21] traditional plant breeding has usually relied on crosses with closely related plants, including wild relatives, because of sexual incompatibility barriers. However, with genetic engineering these barriers do not exist. Genes may be transferred from closely related species, distantly related species or even from organisms outside the plant kingdom such as bacteria, insects and mammals. For example, genes encoding human serum albumin have been transferred to and expressed in potato and tobacco plants.[41]

The problem may not be with the genetically engineered crops themselves but rather with the possibility that they may transfer these genes to wild (weedy) relatives through sexual hybridization. Several crop species are known to be cross-compatible with wild species; e.g., johnson grass (Sorghum halepense) is cross-compatible with cultivated sorghum (Sorghum bicolor). Some crops, including most forage grasses, have undergone relatively little domestication, compared to more intensively cultivated crops, such as maize or soybean. Hence, they possess certain weedy characteristics and are even considered as weeds in some cases. Ellstrand and Hoffman[10] use the example of bermudagrass. This is an important turf and forage species, but it is also, in many areas of the United States and under certain conditions, one of the worst weeds. If a gene for herbicide, salinity or pest tolerance were to be transferred from a cultivated variety into weed populations it could have an immediate economic ecological impact. Herbicide resistance in any forage grass species may receive considerable scrutiny before release into the environment. Therefore, regarding genetic engineering of forage grasses, breeders face not only the slow development of technology, due to lack of commercial interest and financial resources, but also the possibility of greater problems in the ultimate release and use of genetically transformed plants.

References

1. Ahloowalia, B.S., 1976. p. 115-122. In: K. Jones and P. E. Brandham (eds.). Current Chromosome Research. Elsevier/North Holland Biomedical Press, Amsterdam.
2. Ammirato, P., 1983. Bio/Technology 1:68-74.
3. Bingham, E.T., 1979. p. 472-487. In: W.H. Lewis (ed.). Polyploidy: Biological Relevance. Plenum Press, New York.
4. Brill, W.J., 1985. Science 227:381-384.
5. Carlson, P.S., H.H. Smith and R.D. Dearing, 1972. Proc. Nat. Acad. Sci. (U.S.) 69:2292-2294.
6. Conger, B.V., R.N. Trigiano and D.J. Gray, 1988. p. 49-61. In: M.S.S. Pais, et al. (eds.). Plant Cell Biotechnology. NATO-ASI Series H. V. 18. Springer-Verlag, Berlin.
7. De Block, M-,L. Herrera-Estrella, M., Van Montagu, J. Schell and P. Zambrisky, 1984. EMBO J. 3:1681-1689.
8. De Buyser, J., Y. Henry, P. Lonnet, R. Hertzog and A. Hespel, 1987. Plant Breeding 98:53-56.
9. Eizenga, G.C., 1989. Genome 32:373-379.
10. Ellstrand, N.C. and C.A. Hoffman, 1990. Bioscience 40:438-442.
11. Flavell, R.B., 1989. Phil. Trans. R. Soc. Lond. B 324:525-535.
12. Fromm, M.E. et al., 1990. Bio/Technology 8:833-839.
13. Fujii, J.A., D.T. Slade, K. Redenbaugh and K.A. Walker, 1987. Trends in Biotechnol. 5:335-339.
14. Gasser, C.S. and R.T. Fraley, 1989. Science 244:1293-1299.
15. Gordon-Kamm, W.J. et al., 1990. The Plant Cell 2:603-618.
16. Gray, D.J. and B.V. Conger, 1985. Plant Cell Tissue Organ Cult. 4:123-133.

17. Gray, D.J., B.V. Conger and G.E. Hanning, 1984. Protoplasma 122:196-202.
18. Gray, D.J., B.V. Conger and D.D. Songstad, 1987. In Vitro Cell. Devel. Biol. 23:29-33.
19. Gray, D.J. and A. Purohit. Crit. Rev. Plant Sci. (in press).
20. Hauser, V.L., 1983. Trans. ASAE 26:74-78.
21. Hoffman, C.A., 1990. Bioscience 40:434-437.
22. Horn, M.E., R.D. Shillito, B.V. Conger and C.T. Harms, 1988. Plant Cell Rep. 7:469-472.
23. Horsch, R.B., R.T. Fraley, S.G. Rogers, P.R. Sanders, A. Lloyd and N. Hoffman, 1984. Science 223:496-498.
24. Kasha, K.J. and K.N. Kao, 1970. Nature 225:874-876.
25. Kasperbauer, M.J., R.C. Buckner and W.D. Springer, 1980. Crop Sci. 20:103-106.
26. Keeler, K.H., 1989. Bio/Technology 7:1134-1139.
27. Kyozuka, J., T. Kaneda and K. Shimamoto, 1989. Bio/Technology 7:1171-1174.
28. Larkin, P.J. and W.R. Scowcroft, 1981. Theor. Appl. Genet. 60:197-214.
29. Laurie, D.A. and J.W. Snape, 1990. Theor. Appl. Genet. 79:813-816.
30. Matzk, F., 1981. Theor. Appl. Genet. 60:119-122.
31. Morrish, F., V. Vasil and I.K. Vasil, 1987. Adv. Genet. 24:431-499.
32. Paterson, A.H. et al., 1988. Nature 335:721-726.
33. Ratner, M., 1990. Bio/Technology 8:401-403.
34. Redenbaugh, K., D. Slade, P. Viss and J.A. Fujii, 1987. Hortscience 22:803-809.
35. Reed, J.N. and B.V. Conger, 1985. Environ. Exp. Bot. 25:277-284.
36. Rhodes, C.A. et al., 1989. Bio/Technology 7:581-587.
37. Rines, H.W. and L.S. Dahleen, 1990. Crop Sci. 30:1073-1078.
38. Schenk, R.U. and A.C. Hildebrandt, 1972. Can. J. Bot. 50:199-204.
39. Schneider, K., 1990. The New York Times Magazine, June 10, 1990. p. 26-39.
40. Shimamoto, K., R. Terada, T. Izawa and H. Fujimoto, 1989. Nature 337:274-276.
41. Sijmons, P.C., et al., 1990. Bio/Technology 8:217-221.
42. Taliaferro, C.M., S.M. Dabo, E.D. Mitchell, B.B. Johnson and B.D. Metzinger, 1989. Plant Cell Tissue Organ Cult. 19:257-266.
43. Tanksley, S.D., N.D. Young, A.H. Paterson and M.W. Bonierbale, 1989. Bio/Technology 7:257-264.
44. Terada, R., J. Kyozuka, S. Nishibayash; and K. Shimamoto, 1987. Molec. Gen. Genet. 210:39-43.
45. Tomes, D.T., 1990. p. 23-32. In: H.J.J. Nijkamp, et al. (eds.). Progress in Plant Cellular and Molecular Biology. Kluwer Acad. Pub.,Dordrecht.
46. Vasil, I.K., 1990. Bio/Technology 8:296-301.
47. Zenkteler, M., 1990. Crit. Rev. Plant Sci. 9:267-279.

BREEDING PERSPECTIVES IN PERENNIAL FODDER CROPS

C. POISSON

Station d'Amélioration des Plantes Fourragères, I.N.R.A., LUSIGNAN, FRANCE

SUMMARY

For a long time, the target of forage plant breeding has been the best results in the optimum conditions of production . Overproduction and the increase of pollution lead to take up the breeding objectives again.

The use of forage plants is bounded to change, but plant breeding will be able to consider only a few objectives. Among them, a greater attention should be paid to the improvement of adaptative processes, to the growth with moderate fertilization, to the feeding value, and to the adaptation to grassland management.

The use of forage plants as covering plants implies the fulfilment of somme requirements ; but it is possible to find adapted species and varieties among the cultivars already selected for forage purposes. On the contrary their use as fallow plants will require a specific breeding work.
Keywords : fodder-crop, plant breeding, protective crops, cultivated fallow, disintensification.

INTRODUCTION

From the beginning of fodder plant breeding, the object has been to increase the animal production per ha. Simultaneously, agronomy and animal genetics achieved significant advances.

But at the last International Grassland Congress H. VAN DER MEER and W. WEDIN (1), showed that the intensification of the fodder crops and the increase in animal performances lead to overproduction in Western Europ ; at the same time the concentration of high producing animals brings about pollution.

That brings us to question the aims of selection more quickly and more thoroughly than in the past. We have to analyse the inferences of the present state of things, to determine desirable objectives, to look for the corresponding criteria, to evaluate the costs and the risks, and then to infer the feasibility in the short and in the long term.

The stock farming situation vary largely in the European Community according to the pedoclimatic conditions, the animal production and the farms structures. In fact, overproduction hits the whole Community but pollution is the consequence of the most intensive systems only.

The measures adopted may induce a rural depopulation of the less favoured regions. But the Community itself has clearly expressed its will to avoid neglect which drives to waste land.

Similarly, cereals, oil and protein crops, may come the cause of overproduction and harmful effects. On the one hand, soil leaching during the winter, and the resulting nitrogen leakage give a new interest to "protective crops" ; on the other hand, it will be necessary to choose species usable as "filling crops" in places where laying land fallow will be the means of reducing the production.

We have to face two types of problems : first, where stock-farming is the most intensive, how to reduce production and pollution level without reducing the farmer standard of living, knowing that, theoretically at least, the importance of surface as a limiting factor is decreasing ? Secondly, how to use at best less favourable zones so that their maintenance is as inexpensive as possible for the Community, for example through a minimum animal production ?

HOW TO DEFINE THE OBJECTIVES AND WHAT SOLUTIONS TO CHOOSE ?

Arbitrarily and schematically we shall set the favourable against the unfavourable areas, the former being characterized by a rainfall distribution allowing grazing during the greatest part of the year. But, first, we will consider the problems of the protective and filling crops.

The protective crops Their main object is to decrease the nitrate concentration in the soil solution. Consequently they must grow in autumn and in the beginning of winter since at that time the evapotranspiration is low (2). These crops may be sown on bare soil, or under another crop, maize for example, the last case being more troublesome owing to competition for water and light, but also for tolerance to the weed killers of the main crop. Suitable species must have low-price seeds, and also have a rapid growth and be easily destroyed either naturally by freezing or artificially. The decomposition must release nitrogen as slowly as possible (3).

May be there is no species answering perfectly these requirements, but there are many acceptable solutions already used like "alternative" rye-grass, rye, mustard, rape, vetch. Consequently there is no immediate need of a special breeding work.

The cultivated fallow Some species are already used against erosion in the mountains, on ski tracks, or for sowing artificial banks as along motorways, or for turfing in orchards.

If a large part of cultivated soils has to be "uncultivated", we need some species able to maintain and if possible to increase soil fertility and to avoid the extension of land waste (4). They must have an easy rooting, good persistency without fertilization, a high tillering to achieve a satisfactory covering of the soil, good competiting ability against other species, particularly tree species, a good resistance to diseases, frost and summer drought and a good perenniality. At last, the maintenance, either by grazing or mowing, has to be restricted to the minimum, even if it is necessary to insure the perenniality of the plant covering. The species which meet best these requirements must have a weak development, a dense tillering, a fast renewal of the tillers and an unusually neglected character : the fast disappearance of dead organs to prevent damages to the plant covering.

Most of the above described characters are those of a hardy turfgrass, eventually mixed with small size legumes. Some species and varieties have been systematically tested in France by public services (4). Some *FESTUCA RUBRA*, *FESTUCA OVINA*, *FESTUCA PRATENSIS*, *PHLEUM PRATENSE* and *LOLIUM PERENNE* seem well suited to this use. For the near future, the plant breeding has an important role to play and, apart aesthetic characters which are of minor importance in this case, the objectives are very close to those of a turfgrass selection.

Unfavorable areas In the most unfavourable areas, the problems become complicated with the diversity of situations, but generally such are the constraints (excess of water in the soil during the winter, heat and drought in summer) that the fodder production is restricted to a short period in the spring (5).

The condition to maintain an animal production is the possibility to have two areas of fodder production in hand : the greatest part used as an extensive pasture, and under-going eventually some improvements, the other part used in an intensive way for silage or hay production (which is less detrimental for the environment than intentive grazing) (6). The management of this area is expensive but ensiling allows to let grass growth as long as possible. Selected varieties have to combine hardiness and good feeding value : one can think of mixtures of grasses with hardy legumes (7). At the same time the areas devoted to extensive pasture have to be mechanically but simply maintained. As far as possible it is desirable to oversow legumes having a high competiting ability, a quick growth, and able to multiply on site to insure persistency.

There are not new requirements and they can be considered classical though the corresponding criteria are not easy to use. The potential yield seems important only for hay and silage ; in all other cases the adaptability to constraints is the main point.

THE SPECIFIC PROBLEMS LINKED TO THE DISINTENSIFICATION

In the most favourable areas, where is the most intensive stock-farming the consequences of the reduction in animal number per ha have to be foreseen.

The first point deals with the technical and breeding consequences.

The second one is to maintain the farmer standard of living by cutting down the expenses, particularly the labour cost, simplifying the grazing management.

Adaptation to a lower stocking-rate During the last ten years, English studies (8) have shown that, at any time, there is a state of plant covering such as it allows the best equilibrium between the grass consumption and the maintaining of a sufficient leaf area index for photosynthesis which minimizes the losses of production by senescence. This equilibrium depends on the stocking rate and on the grass growth, the latter depending on the season and on nitrogen fertilization.

A. MAZZANTI (9) has shown recently, on continuous grazing sheep on tall fescue, the equilibrium being maintained by the adjustement of the stocking rate, that the effect of nitrogen fertilization is larger on the grass consumption by the animal than on the grass growth, as a result of the reduction of losses due to senescence. A simultaneous reduction in the level of nitrogen fertilization and in stocking-rate entails a more than proportionnal decrease in the animal production through a very significant decrease in the efficiency of grass utilization. Mereover the digestibility of the leaves decreases owing to the ageing. Besides the improvement of the feeding value has an increasing importance. Other trials have shown that it is possible to improve the palatability and digestibility of tall fescue (10). More recent studies show that the increase in soluble sugar leads to the same results in perennial rye-grass (11). For the same reason, a greater attention has to be paid to disease resistance (12). For both characters, feeding value and disease resistance, artificial polyploidy seems a means of increasing their level as shown by many examples, namely on dodecaploid euro-mediterranean fescues, tetraploid rye-grass and red clover. At the same time, plant breeding must have to take a greater interest in grass and legumes mixtures on the one hand and, on the other hand, to the genetic variability in grasses for the best nitrogen utilization. Concerning the mixtures, the interactions between the components have already been well described from a statistical point of view (13). D. EVANS (14) has shown recently that the choice of white clover cultivars influences significantly the dry matter and nitrogen yield of the companion grass. Nevertheless we must admit that much has to be done to understand the physiological relations between the components : for example the effect of light, and the use of soil nitrogen by one or the other partner. At the moment, the breeder has only a few criteria at his disposal, as far as he has any. Yet it seems that new advances can be achieved concerning grazing tolerance in lucerne (15) and I. RHODES (16) has shown that it is possible to select white clover combining close grazing tolerance, satisfactory production and perenniality, using criteria such as behaviour and size of stolons and leaves.

When nitrogen fertilization is under the optimum, the relation between the biomass production and the nitrogen percentage is variable according to genotype. A part is due to differences in metabolic efficiency but another one is certainly related to the ability of roots to extract nitrogen from the soil (17). Whatever the cause, the results of F. LEMAIRE (18) show that the mineral concentration of the nutritive solution has to be adapted to every species to obtain the best yield. To produce the same dry matter yield, in optimal conditions, red fescue has to extract the same quantity of nitrogen from the soil as tall fescue, but it can be satisfied with less nutrient-rich soil. The comparaison of genotype under suboptimal fertilization would probably show more important differences than in optimum conditions. But this will be dificult to make clear, because the soil heterogeneity, hidden in high fertilization level trials, may appear in low nitrogen trials and increase the residual variation so that the differences between genotypes are buffered. P. WILKINS (19) quotes some results on cultivar x nitrogen interaction for the annual dry matter yield perennial rye-grass and concludes that genetic differences in the response to nitrogen exist in the species. Much remains to be done before the plant breeder can use this variability but, in the present circonstances, it is worth trying. Perhaps it would be convenient to use hydroponic systems as A. SCHAPENDONK and A. de VOS (20) suggested in 1987.

Adaptation to grazing system To maintain the best efficiency of grass utilization, we have seen that it is necessary to adjust the stocking rate and the growth of the grass. Nevertheless in disintensified systems it will be more and more difficult to adjust exactly the stocking rate. In these conditions, the adaptability, defined here as the absence of interactions between genotypes and managements, will become at least as important as the potential yield. P. WILKINS (19) stated that in perennial rye-grass there is a variety x harvesting frequency interaction for average annual dry matter yield but, at the same time, he has shown that it is possible to breed for a high yield whatever the harvesting frequency, using only a 6 cuts trial, and selecting the families which have a satisfactory yield at both vegetative and reproductive stape.

These last two years at Lusignan (21-22) has been compared the behaviour of twenty cultivars belonging to three species (*FESTUCA ARUNDINACEA*, *DACTYLIS GLOMERATA* and *LOLIUM PERENNE*), harvested each week at two heights (3 and 8 cm). The dry matter yield and perenniality have been measured on all varieties. Two cocksfoot, two tall fescue, and one rye-grass genotypes have been particularly studied for photosynthesis and morphogenesis. From that, the primary production has been estimated, then the harvest index and the adaptability with regard to the height of harvest. Both tall fescue varieties have a very low adaptability : each one is adapted to specific height of cutting. Applying the unsuited management results either in the disappearance of the grass in one case, or in an important decrease of the harvest index in the other. The rye-grass variety *PREFERENCE* seems particularly suited to a close cutting. When, in orchard grass variety *LUTETIA*, the difference between the harvest indices corresponding to each of the height of cutting is small. For a genotype, the ability to adapt itself seems, among other things, a function of the leaf elongation rate, the tillering ability, the rapidity of adjusting the lamina x sheath ratio, and the angle of tiller to the soil. So, this study shows that there is an inter and intraspecific variability for the adaptation to the height of cutting, related to the morphogenesis of the plant covering.

By another way, the analysis in spaced plants of the offsprings of perennial rye-grass shows that an efficient selection for morphogenesis components is possible, particularly for lamina length, and leaf elongation rate. But the question is whether this selection will be efficient for dense stand conditions.

Then, both experiments point to the possibility of breeding for adaptability, but it is necessary to define precisely the suitable criteria.

CONCLUSION

For a long time, the main object of fodder plant breeding has been to increase the productivity under optimal fertilization conditions. Present circumstances lead not to question the whole of the objectives and criteria, but only to modify their hierarchy. Moreover the fodder species may serve other purposes than animal production. As for all cultivated plants, the improvement of the characters adaptation to pedoclimatic and biotic environment, which allows a better efficiency of inputs, is of increading importance. It will perhaps leads to a more important regionalization than nowadays.

The improvement of the feeding value, what-ever the management, is a major objective.

A great attention must be given on the one hand to the utilization and, on the other hand, to the definition of morphogenetic criteria allowing the improvement of adaptability regarding the different modes of utilization.

Selection is also a business venture whose object is seed production : it is not compatible with an excessive specialization. But it must also be able to adapt as quickly as possible to new trends whose frequency becomes higher from year to year. Trough money and time and work consuming, the maintainance of a plant material as varied as possible seems essential.

REFERENCES

(1) **VAN DER MEER H.G.and WEDIN W.F., 1989.** Present and future role of grass lands and fodder crops in temperate countries with special reference to over production and environment. *XVI International Grassland Congress*, Nice, France, 1711-1718.

(2) **LEMAIRE G..** Personal communication.

(3) **BAYLE B. et CHARPENTIER F..** Personal communication.

(4) **BOURGOIN B., ARNAL G., DINGER F., 1989.** Paysages, occupation et protection des sols : rôle de la prairie, matériel végétal disponible et à créer. *Fourrages*, 119, 321-331.
(5) **GENETTAIS T. et MARQUES V., 1990.** Etude de systèmes fourragers dans les exploitations spécialisées en production ovine du Montmorillonnais. *Mémoire* de DAA, INA Paris-Grignon, 75 p.
(6) **SIMON S.C., et al., 1989.** Rôle agronomique de la prairie dans la gestion du draînage des nitrates vers la nappe phréatique. *Fourrages*, 119, 227-241.
(7) **GAYRAUD P., 1989.** Quelques exemples de prairies graminées-légumineuses adaptées aux zones de climat océanique. *Fourrages*, 119, 299-309.
(8) **PARKER J., 1987.** Systèmes de pâturage et d'utilisation des prairies en Grande-Bretagne. *Fourrages*, 111, 299-314.
(9) **MAZZANTI A., 1990.** Effet de l'azote sur la croissance de l'herbe d'une prairie de fétuque élevée et son utilisation par des moutons en pâturage continu. *Thèse de doctorat es-sciences*, Université Paris-Sud, Centre d'Orsay.
(10) **EMILE J.C., GILLET M., HUGUET L., 1989.** Etude comparative de 2 variétés de fétuque élevée de qualité différente conduites en pâturage continu avec vaches laitières. *XVI Congrès International des Herbages*, Nice, France, 1135-1136.
(11) **WILKINS P. and HUMPHREYS M., 1989.** Breeding for nutritive value, nitrogen use efficiency and yield consistency in perennial rye-grass. *Report* of I.G.A.P., p. 9.
(12) **VAN WIJK A.J.P., 1990.** Personal communication.
(13) **GALLAIS A., 1990.** Théorie de la sélection en amélioration des plantes. *Masson ed.*, p. 79-84.
(14) **EVANS D., 1989.** Variation in grass yield in clover grass mixtures. *Report* of I.G.A.P., p. 14.
(15) **C. POISSON, 1989.** Perspectives de sélection pour les prairies semées. *Fourrages*, 119, pp. 253.267.
(16) **RHODES I., 1989.** White clover breeding. *Report* of I.G.A.P., p. 13.
(17) **LEMAIRE G., et al., 1987.** Ecophysiological approach to plant growth. Consequences for breeding strategies for forage species in contrasting conditions and different managements. *Proc. of Eucarpia Fodder Crops Section Meeting*, 103-118.
(18) **LEMAIRE F., 1987.** Comparaison des besoins en éléments minéraux de différentes espèces de graminées à gazon. *Agronomie* 5 (1) 39-46.
(19) **WILKINS P.W., 1987.** Genotype x management interactions for plot dry matter yield in *LOLIUM PERENNE*. *Proc. of Eucarpia Fodder Crop Section Meeting*, 153-160.
(20) **SHAPENDONK A. and DE VOS A., 1987.** A selection strategy for persistancy testing of grasses on hydroponics. *Proc. of Eucarpia Fodder Crop Section Meeting*, 193-202.
(21) **DJIPDOM J., 1989.** Recherche de critères de croissance d'un gazon de faible hauteur chez une gamme de variétés de ray-grass anglais. *Mémoire* ENITHP, Angers, 36 p.
(22) **HAZARD L., 1990.** Etude de l'adaptation de trois espèces de graminées fourragères (*DACTYLIS GLOMERATA, FESTUCA ARUNDINACEA, LOLIUM PERENNE*) à différentes hauteurs de coupes fréquentes, morphogénèse et échange de CO_2. *Mémoire* de DEA, Université d'Angers, 43 p.

NUTRITIONAL QUALITY OF HERBAGE IN RELATION TO ANIMAL PRODUCTION

A.M. van Vuuren

Research Institute for Livestock Feeding and Nutrition
P.O. Box 160, 8200 AD Lelystad, Netherlands

Summary

To increase the efficiency of animal production, rations should be evaluated according to their potential to produce nutrients for the synthesis of milk fat, milk protein and lactose.

For optimum nutrient supply on herbage-based diets, the protein content in herbage should be 140-170 g/kg dry matter (DM), soluble carbohydrates 80-120 and cell wall content 550-700 g/kg DM. Cell walls should be highly digestible, but the rate of digestion of organic matter in the forestomachs should not exceed 8% per hour.
Keywords: feeding, fermentation, digestion, nutrient supply.

Introduction

At present, feedstuffs, including herbage, are evaluated according to their potential to meet the energy, protein and mineral requirements of the animal (van der Honing & Alderman, 1988). However, such feeding systems take no account of the composition of the feed supplying the energy, with the exception of the energy provided by proteins. With increased animal production more emphasis is put on the supply of specific nutrients required for the synthesis of macro-molecules such as (milk) protein, (milk) fat, lactose and immunoglobulins. Therefore, in addition to the energy, the potential to supply specific compounts for the synthesis of animal tissue and products should be considered more precisely. This will result in an increase of the efficiency of animal production and in a decrease of (nitrogen) losses by faecal and urinary excretion. In future, rations for dairy cows should therefore be evaluated according to their supply of nutrients available to the animal for the synthesis of milk fat, milk protein and lactose.

Until recently, young, highly-digestible herbage was considered an ideal feed for dairy cows. However, research during the last decade has shown that the nutrient supply of grazing dairy cows is insufficient for milk productions above 30 kg/day (Beever & Siddons, 1986; van Vuuren *et al.*, 1986) and results in extensive losses of nitrogen via urinary excretion (van Vuuren & Meijs, 1987).

Supply of organic nutrients

Organic nutrients for ruminants can be divided into ketogenic, glucogenic and aminogenic nutrients which can be used as precursors for the synthesis of lipids, lactose and proteins, respectively (Tamminga & van Vuuren, 1988). Fatty acids (ketogenic) and amino acids (aminogenic) are absorbed from the gastro-intestinal tract, whereas most of the required glucose is derived by gluconeogenesis from lactate and glucogenic fatty acids (mainly propionic acid) and amino acids.

Acetic and butyric acid are the main ketogenic nutrients. These short-chain fatty acids (SCFA) are formed by fermentation of carbohydrates in the forestomachs. Other ketogenic nutrients are long-chain fatty acids (LCFA) either absorbed directly from the gastro-intestinal tract or provided by lipolysis in body fat tissue.

Also the glucogenic SCFA (propionic and valeric acid) are endproducts of fermentation; lactate may either originate from fermentation or be produced by active sceletal muscle.

Amino acids are absorbed from the intestinal tract and originate either from unfermented feed protein or from micro-organisms leaving the forestomachs with the digesta.

Fermentation

In ruminants feed particles are retained in the forestomachs with a mean retention time of 18-24 hours. Here the ingested feed is converted by a fermentative degradation by bacteria, protozoa and fungi. This fermentation alters nutrient composition (Fig. 1). For instance, (glucogenic) carbohydrates are fermented into (ketogenic) acetic and butyric acid and (glucogenic) propionic acid. Microbial fermentation yields ATP, SCFA and ammonia, which may be used as substrates for the synthesis of (aminogenic) microbial protein.

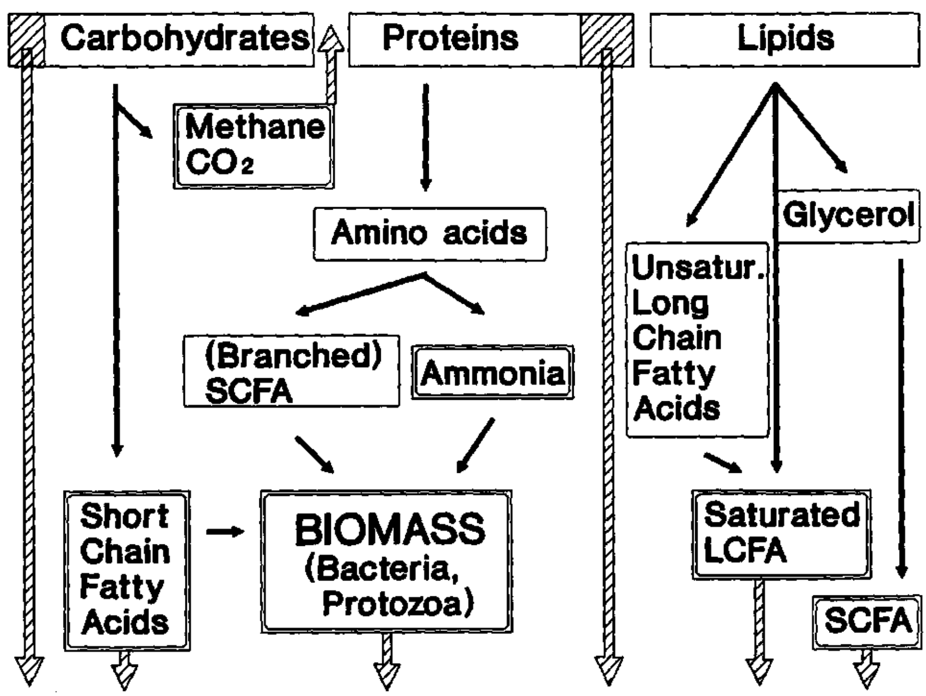


Figure 1. Schematic pathways of fermentative breakdown of carbohydrates, proteins and lipids in the forestomachs of ruminants.

The surplus of SCFA is absorbed and used by the host-animal as ketogenic and glucogenic nutrients. If the rate of fermentation *viz.* rate of acid production exceeds the rate of absorption, the pH in the forestomachs will decrease. Although this will lead to an increase in SCFA absorption, low pH has a negative effect on the activities of cell wall degrading micro-organisms. To prevent high peaks in concentrations of SCFA, organic matter (OM) should not contain high concentrations of rapidly fermentable carbohydrates. However, herbage containing less than 100 g water-soluble carbohydrates per kg DM may be difficult to preserve as grass silage. The OM should be fermented at a rate less than 8% per hour. Ammonia is partly absorbed through the stomach wall and converted into urea by the liver. Urea either re-enters the forestomachs or will be excreted in the urine.

It was assumed that diurnal variations in the concentrations of fermentation products were small in grazing animals, mainly due to the assumed constant feed intake. However, recent experiments showed that diurnal variation in grazing animals is of the same extent as in animals on high-grain winter rations (van Vuuren *et al.*, 1986, Dillon *et al.*, 1989).

Aminogenic nutrients

Proteins in herbage are rapidly fermented in the forestomachs. Results obtained by *in sacco* techniques indicate that the rate of protein degradation of *Lolium perenne* may range between 8 and 13% per hour (van Vuuren *et al.*, 1991). Thus, the ammonia-concentration in the forestomachs of cows consuming good quality herbage, is high (van Vuuren *et al.*, 1986) and, subsequently, only a small proportion of herbage protein enters the small intestine (Beever & Siddons, 1986).

Generally, (soluble) cell contents are fermented more rapidly than (insoluble) cell walls. Consequently, the extent of protein breakdown in the forestomachs is influenced by the ratio cell contents:cell wall in the herbage. Thus, crops with a high content:wall ratio, such as highly fertilized herbage or harvested at early stage of (re)growth are characterized by an extensive breakdown of protein (van Vuuren *et al*,. 1991).

The higher protein content in cell contents and the high rate of fermentation of OM (including protein) also results in a low production of microbial protein (Demeyer & Tamminga, 1986). Thus, the increase in protein concentration realized by cultivation techniques, has only minor effects on the supply of aminogenic nutrients to the animal. This results in low efficiencies of nitrogen utilization and substantial losses of nitrogen. To prevent these losses in the forestomachs the ratio between fermented N and fermented carbohydrates should be *c.* 25 g N/kg carbohydrate (Czerkawski, 1986). In highly fertilized *Lolium perenne* this ratio varies between 30 and 150 (van Vuuren *et al.*, 1990)

Ketogenic and glucogenic nutrients

Roughages, high in cell walls and slowly fermented in the forestomachs, usually yield high proportions of ketogenic nutrients. In high-roughage diets, the ketogenic:glucogenic ratio of SCFA is *c.* 5.0 (van Vuuren, unpublished). However as the rate of fermentation in the forestomachs increases this ratio becomes lower (Murphy *et al.*, 1982). Van Vuuren *et al.* (1986) observed a ketogenic:glucogenic ratio of SCFA of 4.4, in cows consuming fresh, highly fertilized herbage (*L. perenne*). The lower ketogenic:glucogenic ratio seems better attuned to the ratio of required nutrients of the animal, but a ketogenic:glucogenic ratio below 4.0 may lead to a decrease of milk fat secretion, coinciding with an increase in body fat synthesis.

The high rate of fermentation can lead to a decrease in pH and inhibition of cell wall fermenting micro-organisms. This not only hampers the production of ketogenic nutrients from cell walls, but also limits herbage intake.

Herbage intake

The daily intake of OM is the major factor influencing the nutrient supply of dairy cows. Intake of OM of fresh herbage exceeds the intake of conserved forages, like hay and silages. In grazing dairy cows, Meys (1981) observed a maximum organic matter intake of 18.6 kg/day. However, OM intakes observed on mixed rations, containing roughage and concentrates may exceed 20 kg/day (de Visser *et al.*, 1990).

Intake is regulated by physiological control, physical limitations of the gastro-intestinal tract and psychogenic (environmental) factors. The predominating factors limiting herbage intake, are still unknown. Factors that could be important in this respect are the low DM content and the high cell contents, resulting in a high osmolality in the forestomachs and a high production rate of SCFA.

Herbage composition

High-fertilized herbage is characterized by a high rate of OM fermentation, including protein. This leads to a low supply of aminogenic nutrients, an inefficient utilization of feed protein and a high excretion of nitrogen in urine. With low-fertilized herbage the loss of herbage protein is lower, but cell wall digestibility, and hence OM intake, may also be decreased. Low-fertilized herbage often contains high concentrations of rapidly fermentable water-soluble carbohydrates, resulting in low pH-values in the forestomachs.

It is concluded that from a nutritional point of view, ideal herbage should contain 140-170 g protein/kg DM and approx. 100 g water-soluble carbohydrates/kg DM. The remaining DM should mainly consist of cell walls (550-700 g/kg DM). These cell walls should be highly digestible.

References

Beever, D.E. & R.C. Siddons, 1986. Digestion and metabolism in the grazing ruminant. In: Milligan, L.P., W.L. Grovum, & A. Dobson (Eds.): *Control of digestion and metabolism in ruminants*. Prentice-Hall, Englewood Cliffs, NJ. p. 479-497.

Czerkawski, J.W., 1986. An introduction to rumen studies. Pergamon Press, Oxford, 236 pp.

Demeyer, D.I. & S. Tamminga, 1987. Microbial protein yield and its prediction. In: Jarrige, R. & G. Alderman (Eds.): *Feed Evaluation and Protein Requirement Systems for Ruminants*. EUR 10657 EN, Commission of the European Communities, Luxembourg. p. 129-144.

de Visser, H., P.L. van der Togt & S. Tamminga, 1990. Structural and non-structural carbohydrates in concentrate suppements of silage-based dairy cow rations. 1. Feed intake and milk production. *Netherlands Journal of Agricultural Science* 38:487-498.

Dillon, P., G. Stakelum & J.J. Murphy, 1989. The effect of level of herbage intake and concentrate type on rumen fermentation pattern, *in situ* herbage degradability and blood metabolite levels in lactating dairy cows. In: *Proceedings XVI International Grassland Congress, Nice, France*. p. 1155-1156.

Meijs, J.A.C., 1981. Herbage intake by grazing dairy cows. Thesis, Agricultural University, Wageningen, 264 pp.

Murphy, M.R., R.L. Baldwin & L.J. Koong, 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. *Journal of Animal Science* 55:441-421.

Tamminga, S. & A.M. van Vuuren, 1988. Formation and utilization of end products of lignocellulose degradation in ruminants. *Animal Feed Science and Technology* 21:141-159.

van der Honing, Y. & G. Alderman, 1988. Feed evaluation and nutritional requirements. 2. Ruminants. *Livestock Production Science* 19:217-278.

van Vuuren, A.M. & J.A.C. Meijs, 1987. Effects of herbage composition and supplement feeding on the excretion of nitrogen in dung and urine by grazing dairy cows. In: H.G. van der Meer *et al.* (Eds.): *Animal Manure on Grassland and Fodder Crops*. Martinus Nijhoff, Dordrecht. p. 17-25.

van Vuuren, A.M., C.J. van der Koelen, C.J. & J. Vroons-de Bruin, 1986. Influence of level and composition of concentrate supplements on rumen fermentation patterns of grazing dairy cows. *Netherlands Journal of Agricultural Science* 34:457-467.

van Vuuren, A.M., S. Tamminga & R.S. Ketelaar, 1990. Ruminal availability of nitrogen and carbohydrates from fresh and preserved herbage in dairy cows. *Netherlands Journal of Agricultural Science* 38:499-512.

van Vuuren, A.M., S. Tamminga & R.S. Ketelaar, 1991. *In sacco* degradation of organic matter and crude protein of fresh grass (*Lolium perenne*) in the rumen of grazing dairy cows. *Journal of Agricultural Science, Cambridge* (in press).

THE ROLE OF LEGUMES IN GRASSLANDS AND FORAGE PRODUCTION

L.'t Mannetje

Department of Field Crops and Grassland Science, Agricultural University, Wageningen, The Netherlands

Summary

The main grassland and forage legumes in western Europe are *Trifolium repens* (white clover), *T.pratense* (red clover) and *Medicago sativa* (lucerne), but they are not used much in intensive farming. The dry matter yield of experimental *T.repens*-grass pastures ranges from 5 to 15 tons/ha/year. The yield of *T.pratense* is between 6 and 15 tons and that of *M.sativa* between 13 and 18 tons DM/ha/year. Animal production from *T.repens* based pastures can be 80% and that from *M.sativa* and *T.pratense* can equal that of grass pastures fertilised with 400 kg N/ha/year. However, the main problems with *T.repens* are its rapidly declining yield and poor persistence in pastures, which may be due to inadequate genotypes, management, pests and diseases. Furthermore, *T.repens* has poor spring growth and its performance is generally inconsistent. *T.pratense* can be productive from 1.5 to 3 years, whereas *M.sativa* may last 4 years. However, both crops also suffer from pests and diseases as well as from weed infestation and wheel damage. All three legumes can cause fatal bloat in cattle.

T.repens-based pastures have a place in situations in which the use of N fertiliser is prohibited or restricted. Research, further plant breeding and cultivar development will be necessary to improve on the presently available cultivars. *T.pratense* and *M.sativa* can be used as forage crops for silage or artificial drying on animal and crop farms.

Introduction

Legumes are important to agriculture, because they supply their own nitrogen (N), enrich the N content of the soil and have a high nutritive value. Legumes also supply associated grasses with N as a result of the mineralisation of accumulated organic N in the soil. Thus, legumes can contribute to the conservation of energy by reducing the need for N fertiliser.

The main forage legumes for temperate climates are species of *Trifolium* and *Medicago*. Some global statistics indicate the significance of legumes for forage production in regions with relatively extensive farming systems (Marten et al. 1989). There are some 13 Mha of *M.sativa*, 5 Mha of *T.repens* and 6 Mha of *T.pratense* in the USA. Other countries with large areas of forage legumes are Australia with 6 Mha of *T.repens* and 17 Mha of *T.subterraneum*. New Zealand has 9 Mha of *T.repens* and it has been estimated that it exports about 4400 tons of *T.repens* seed annually (Frame and Newbould 1986).

The estimated annually sown area of *T.repens* in the United Kingdom was about 184 Kha in 1982, but only 144 Kha in 1989. This decline happened despite serious efforts by research establishments to show the benefits and best management of clover pastures. Over the same period, *T.pratense* seed sales have plummeted from 425 tons in 1980 to 77 tons in 1989 in the UK (Sheldrick 1990).

In The Netherlands the percentage of sales of pasture seed mixtures containing T.repens has declined from 62 in 1969/70 to 3% in 1988/89 (Anon. 1990). The estimated area of T.repens pastures sown in The Netherlands is about 8 Kha/year. T.pratense use in The Netherlands is negligible.

M.sativa sowings in The Netherlands declined from 6.6 Kha in 1968 to 2.0 Kha in 1982, but has since increased again to 5.3 Kha in 1989. Most of this area is on crop farms for artificial drying for the compound feed industry.

This paper will be restricted to T.repens, T.pratense and M.sativa in western Europe.

Productivity of forage legumes

T.repens

Ennik (1982) showed that the maximum yield obtained in The Netherlands on 1 - 2 year old pure T.repens swards, without N fertiliser, was 8 tons DM/ha/annum. With an increasing proportion of grass in a mixture with T.repens, as a result of increasing N fertilisation, the DM yield increased to 13 tons/ha/annum at the point where all the T.repens had been replaced by grass. With 50% T.repens in the mixture the total DM yield would be 10 tons DM/ha/annum. On three year old plots the Louis Bolk Institute in The Netherlands measured 5.6 tons DM/ha with the mixture BG5 (containing 13% T.repens) and 13.3 tons DM/ha with a New Zealand mixture containing T.repens and T.pratense without N fertiliser (T. Baars and M. van Dongen, pers. com.).

In a survey between 1975 and 1983 of National List Trials in the United Kingdom DM production of T.repens-based experimental swards ranged from 2.0 to 15.5 t/DM/ha/annum (Frame and Newbould 1986).

The DM productivity reflects the amount of N fixed, which has been estimated at between 65 - 280 kg/ha/year in western Europe (Frame and Newbould 1986).

T.repens has a superior nutritive value than Lolium perenne, expressed in terms of cell wall content and voluntary intake. The digestibility of T.repens declines more slowly than that of L.perenne with increasing maturity. Animals fed pure clover or increasing proportions of clover in mixtures with grass produced more milk and liveweight gain than those fed pure perennial ryegrass (Thomson 1984). In England, Stewart (1984) reported that grass pastures receiving 265 kg N/ha/year supported 61% more cows, produced 65% more milk and was 159% more profitable during a five year period than clover based pastures, which had a high T.repens content.

Ryan (1986) reported from Ireland that milk production/cow was 6% higher, but per ha 15% lower on T.repens-based pastures without N fertiliser than on grass pastures fertilised with 400 kg N/ha/year over a three year period. Similar results were reported by Gately (1982).

Stewart (1984) concluded that under farm conditions in the UK grass-clover swards could support comparable stocking rates (about 1500 kg liveweight/ha) and produce as much liveweight gain (800 kg/ha/year) as N fertilised grass receiving 230 kg N/ha.

In a sheep grazing experiment in the UK, Orr et al.(1990) found that with a small and declining percentage of T.repens in the mixture, liveweight gains could be maintained that were 68 to 82% of those of a grass sward fertilised with 420 kg N/ha/annum over three years. Low clover contents with less than 65 kg N fixation/ha/year appear to be able to sustain acceptable liveweight gains in sheep as was shown by Sheehy

(1989) in a model calculation.

T.pratense

T.pratense (red clover) is better adapted to acid soils than M.sativa and mainly used for silage and artificial drying. The crop yields between 6-10 tons DM/ha/year, although experimental yields of 16 tons DM/ha in the first year have been measured (Young 1984). T.pratense has a high feeding value and sheep and cattle eat more of its silage than of grass silage. Short term animal production on grazed T.pratense was similar to that on fertilised L.perenne (Campling 1984).

M.sativa

M.sativa (lucerne) is well adapted to the climate of western Europe. In The Netherlands the DM yield without N fertiliser ranged from 13 to 18 tons/ha/annum (Sibma and Spiertz 1985). Also in the UK lucerne yields, without N fertiliser, about 15 tons/DM/ha/year (Aldrich 1984). On acid sandy soils good results can be obtained when the seed is lime-pelleted before sowing (Deinum and Eleveld 1986, Schröder 1988).

Lucerne's nutritive value is characterised by comparatively lower cell wall contents, higher cell solubles, crude protein, minerals and lignin contents than L.perenne (Spedding & Diekmahns 1972, Thom & Smith 1980). Despite a lower organic matter digestibility of lucerne than of L.perenne, intake of lucerne is 20 - 30% higher than that of grass and this has been attributed to the faster rate of digestion of the lucerne (Demarquilly 1976, Deinum & Hof 1984). Campling (1984) concluded that both sheep and cattle grew appreciably faster on lucerne than on grass, whilst milk production of cows was similar.

Shortcomings of forage legumes

It is evident that T.repens , T.pratense and M.sativa are productive forages. However, the reported production figures all refer to short term experiments.

Frame and Newbould (1984) reported for the United Kingdom that farmers make little use of white clover and that the T.repens content and the DM production of these grasslands were very low. The main reasons offered were that farmers are not always able to maintain the optimal grazing and cutting management for clover grasslands and that they use too much N fertiliser.

The main disadvantages of forage legumes are: 1) they are less predictable and have a lower yield, particularly in spring, than grass with N fertiliser, 2) they lack persistence, 3) they can cause bloat (tympanitis), 4) their small-seededness make them difficult to establish (Frame and Newbould 1986, Laidlaw and Frame 1988). To overcome the low early spring production of clover-based grasslands, farmers often apply up to about 100 kg N/ha before the first grazing. This has a deleterious effect on clover growth.

The unpredictable yield and lack of persistence of T.repens have been variably attributed to inadequate management, adverse effects from soil organisms, slugs, grubs and diseases (Frame & Newbould 1986). Of these the clover cyst nematode (Heterodera trifolli) is widespread in western Europe and causes much damage, whilst the stem eelworm (Ditylenchus dipsaci) and root knot nematode (Meloidogyne hapla) are also noted pests. In addition, there are several fungus diseases, such as clover rot, caused by Sclerotina trifoliorum and root deterioration caused by

Fusarium spp. which contribute to loss of production and persistency.

T.repens spoils its own habitat by accumulating N in the rhizosphere, which encourages growth of grasses and weeds, which leads to acidification of the soil (Gramshaw et al. 1989) and by the attraction of soil born pests and diseases.

T.pratense and M.sativa are generally used as short term forage crops. T.pratense has a productive life of 1.5 to 3 years, whilst M.sativa can last for 4 years (Aldrich 1984). The lack of persistence of these forage crops is not felt as a particular disadvantage. Nevertheless, there are several diseases which attack these species and cause reduced yields and persistency. In addition, the crops suffer from weed infestation and wheel damage (Aldrich 1984).

Breeding of T.repens

The lack of persistence and low and inconsistent dry matter yield of T.repens may be partly caused by pests, diseases and competition from grasses. Plant breeding efforts and cultivar development by the Welsh Plant Breeding Station have had the objectives of increased compatibility between the clover and grass, pest and disease tolerance, stolon survival over winter and better spring growth. New cultivars have been produced over the last few years, which offer scope for improvement on the listed shortcomings (Rhodes 1984, Evans et al. 1990). Further research and breeding work are required to identify plant characteristics which are correlated with persistence, early spring growth and compatibility with grasses. Management also has a big effect on clover performance and persistence and together with new cultivars much field research is required to devise optimal management techniques.

Place of forage legumes in farming practice

T.repens would have a place in permanent and ley pastures in farm systems which operate without or at low levels of N input. These exist in extensive farm systems for beef and sheep production in hill country e.g. in central Europe and the United Kingdom and in so called ecological/biological/alternative farming. There also is a place for T.repens pastures in environmentally sensitive areas (e.g for water harvesting) and areas designated for nature conservation in combination with grassland farming. Without the use of N fertiliser, pastures in western Europe not only produce less dry matter, but also low quality feed, inadequate for highly productive dairy cows (Korevaar 1986). T.repens-based pastures can yield up to 70% of the dry matter yield of pastures fertilised with 400 kg N/ha/year and produce high quality feed. However, T.repens still has to be proven to be persistent for more than 4 to 5 years. The attractiveness of T.repens-based pastures from an environmental point of view is its relatively low N input. Improvement of T.repens cultivars and of management for optimal clover content of pastures will detract from the environmental advantage as more N will be put into circulation, which is just as liable to leakage to the environment as fertiliser N, particularly under grazing ('t Mannetje and Jarvis 1990).

T.pratense and M.sativa forage crops can be used in animal as well as in arable farming. In the latter case farmers need to have an outlet for their product which needs to be dried before it can be sold to compound feed manufacturers. There are not enough drying plants available in arable farming regions.

References

Aldrich, D.T.A., 1984. Lucerne, red clover and sainfoin-herbage production. In: D.J. Thompson (Ed.): Forage legumes. British Grassland Society Occassional Symposium No. 16, p. 126-131.

Anonymous, 1990. 65e Beschrijvende rassenlijst voor landbouwgewassen 1990. RIVRO, Wageningen, The Netherlands.

Campling, R.C., 1984. Lucerne, red clover and other forage legumes: feeding value and animal production. In: D.J. Thompson (Ed.): Forage legumes. British Grassland Society Occasional Symposium No. 16, p. 140-146.

Deinum, B. & J. Eleveld, 1986. Effects of liming and seed pelleting on the growth of lucerne (Medicago sativa L.) on sandy soils. In: F.M. Borba & J.M. Abreu (Eds.) Proceedings 11th General Meeting European Grasslands Federation, Troia, Portugal, p. 270-273.

Deinum, B. & G. Hof, 1984. Benutting en voederwaarde van luzerne en rode klaver. Gebundelde Verslagen Nederlandse Vereniging voor Weide- en Voederbouw Nr. 25, p. 45-56.

Ennik, G.C., 1982. De bijdrage van witte klaver aan de opbrengst van grasland. Landbouwkundig Tijdschrift 94: 363-369.

Evans, D.R., T.A. Williams & S.A. Mason, 1990. Contribution of white clover varieties to total sward production under typical farm management. Grass and Forage Science 45: 129-134.

Frame, J. & P. Newbould, 1986. Agronomy of white clover. Advances in Agron. 40: 1-88.

Gately, T.F., 1982. Evaluation of the role of white clover (cv. Blanca) for milk production. Grass and Forage Science. 37: 171-172.

Gramshaw, D., J.W. Read, W.J. Collins & E.D. Carter, 1989. Sown pastures and legume persistence: an Australian overview. In: Marten, G.C., A.G. Matches, R.F. Barnes, R.W. Brougham, R.J. Clements & G.W. Sheath (Eds.). Persistence of forage legumes. American Society of Agronomy, Inc., Crop Science Society of America, Inc. Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Korevaar, H. (1986). Produktie en voederwaarde van gras bij gebruiks- en bemestingsbeperkingen voor natuurbeheer. Doctorate Thesis, Agricultural University, Wageningen, Report no. 101 Experimental Station for Cattle, Sheep and Horse Husbandry, Lelystad.

Mannetje, L.'t and S.C. Jarvis, 1990. Nitrogen flows and losses in grazed grasslands. Proceedings 13th European Grasslands Federation Meeting, Banská Bystrica, Czechoslovakia, 1: 114-131.

Marten, G.C., A.G. Matches, R.F. Barnes, R.W. Brougham, R.J. Clements & G.W. Sheath (Eds.) 1989. Persistence of forage legumes. American Society of Agronomy, Inc., Crop Science Society of America, Inc. Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Orr, R.J., A.J. Parsons, P.D. Penning, & T.T. Treacher, 1990. Sward composition, animal performance and the potential production of grass/white clover swards continuously stocked with sheep. Grass and Forage Science 45: 325-336.

Rhodes, I. 1984. Herbage legume breeding at the Welsh Plant Breeding Station. In: D.J. Thompson (Ed.): Forage legumes. British Grassland Society Occasional Symposium No. 16, p. 61-65.

Ryan, M. (1986)., Milk output from white clover-based versus nitrogen-based dairy systems. In: F.M. Borba & J.M. Abreu (Eds.) Proceedings 11th General Meeting European Grasslands Federation, Troia, Portugal, p. 301-304.

Schröder, J., 1988. Teelt van luzerne. PAGV Teelthandleiding nr. 25, Lelystad, The Netherlands (35 pp).

Sheehy, J.E. 1989. How much dinitrogen is required in grazed grasslands? Annals of Botany 64: 159-161.

Sheldrick, R.D., 1990. Do clovers have a future? Grass Farmer No. 35: 10-12.

Sibma, L. & J.H.J. Spiertz, 1985. Dry matter production and nitrogen utilization in cropping systems with grass, lucerne and maize. I. Comparison of crop characteristics, growth and production. Netherlands Journal of Agricultural Science 34: 25-35.

Spedding, C.R.W. & E.C. Diekmahns, 1972. Grasses and legumes in British agriculture. Commonwealth Agricultural Bureaux, Farnham Royal, England.

Stewart, T.A., 1984. Utilising white clover in grass based animal production systems. In: D.J. Thompson (Ed.): Forage legumes. British Grassland Society Occasional Symposium No. 16, p. 93-103.

Thom, E.R. & G.S. Smith, 1980. Effect of stage of growth and season on the major element composition of the aerial parts of lucerne (Medicago sativa L.). Proceedings Agronomy Society of New Zealand, 10: 23-26.

Thomson, D.J., 1984. The nutritive value of white clover. In: D.J. Thompson (Ed.): Forage legumes. British Grassland Society Occasional Symposium No. 16, p. 78-92.

Young, N.R., 1984. Red clover for conservation on upland farms. In: D.J. Thompson (Ed.): Forage legumes. British Grassland Society Occasional Symposium No. 16, p. 132-135.

THEME 2

NOVEL STRATEGIES AND BIOTECHNOLOGY

Posters

Identification of Components of Chance-hybrids in Lolium multiflorum Lam. by using Electrophoresis

U. Bertling and G. Kobabe

Institute of Agronomy and Plant Breeding, University, Von-Siebold-Str. 8, D-3400 Göttingen, Germany F.R.

Summary

An approach is described to determine the portion of the hybrid component in a chance-hybrid. The genotype frequencies are found out by electrophoresis. A ratio f_v following the Hardy-Weinberg law is calculated. Reasons for introducing labelled parents are discussed.
Keywords: chance-hybrid, competitive ability, electrophoresis, labelled population.

Introduction

A chance-hybrid produced by open pollination of two parents which are self fertile and outbreeding consists of three components, i.e. the hybrids (interpopulational offsprings) and the offsprings from crossing within the parents (intrapopulational offsprings). In an ideal situation the hybrids will be 50% of the seed harvest. When this blend is sown in the field the initial composition may change because of competition between the components. Two experiments are described in which a method by means of electrophoresis is investigated to observe the shift of the components in the field.

Results and discussion

Experiment 1

In 1989 a field trial with three chance-hybrids produced by open pollination of two inbreds each was carried out. The chance-hybrids were sown in six rows two metres long per plot. Samples were taken by cutting grass stalks along the rows. The leaves of each stalk were used to determine the genotype of isozyme PGI-2 by starch gel electrophoresis. The genotype frequencies of the chance-hybrids (I, II, III) were determined (table 1).

In a first step to analyse the frequencies the attempt was made to compare the observed distribution with a calculated one in order to draw conclusions on the frequencies of the hybrids. Based on the Hardy-Weinberg law the factor f_v was calculated:

$$f_v = \frac{p_{ab}}{2 * sqr(p_{aa} * p_{bb})}$$

p_{ab} genotype frequency of ab
p_{aa} genotype frequency of aa
p_{bb} genotype frequency of bb

$f_v > 1.0$ means that there are more heterozygous genotypes than expected.

In many cases a surplus of heterozygous genotypes was found. But it was not possible to put this surplus down to a higher competitive ability of the hybrids. Other effects which disrupt Hardy-Weinberg frequencies, e.g. self incompatibility, may be responsible. The parents of each chance-hybrid were analysed, too (table 1) and the results support this explanation.

The frequencies of the parents showed that the hybrids could have a heterozygous as well as a homozygous genotype. So it was not possible to distinguish the intrapopulational offsprings from the hybrids and to derive the percentage of hybrids indirectly by Hardy-Weinberg.

Moreover, Hardy-Weinberg does not consider the different tillering. Because of heterosis the hybrids could be expected to have more stalks than the intrapopulational offsprings. Therefore the contribution of the components to the sample might not be the same.

The best way to determine the outcrossing rate directly and to become independent of Hardy-Weinberg is to use electrophoresis in order to label the components.

Experiment 2

This year the seeds of the first chance-hybrids (A, B) produced by labelled inbreds in isolation tents were harvested. The genotype frequencies of the three components and the outcrossing rate are shown in table 1.

For the next field trial it has to be noticed that the outcrossing rate is determined by plants while in the field stalks are sampled. Therefore the outcrossing rate might not be equal to the initial composition of the chance-hybrid in the field. The tillering must be taken into account.

Table 1. Genotype frequencies (in percent) of chance-hybrids and parents (1989) and chance-hybrids (1990)

	parents			parents			parents				
	420	611	I	550	443	II	502	735	III	A	B
aa	0	0	0	0	17	1	0	0	0	0	0
ab	1	0	1	0	32	18	0	0	0	0	0
ac	0	0	0	0	0	2	0	0	0	0	0
ad	0	0	1	0	14	5	0	0	0	0	0
bb	13	1	4	77	23	29	16	4	11	0	57
bc	52	13	20	8	0	7	29	0	13	0	0
bd	14	7	23	13	13	28	19	53	39	0	39
cc	12	4	11	0	0	1	11	0	0	44	0
cd	7	57	30	1	0	2	19	5	20	35	0
dd	0	18	10	0	1	7	6	38	17	21	4
aa	0	0	0	0	12	2	0	0	1	0	0
ab	1	0	2	0	23	60	0	0	0	0	0
bb	11	1	13	64	16	95	13	3	37	0	93
fv	...	...	...	...	0,8	2,2	...	...	0,0	...	...
aa	0	0	0	0	12	2	0	0	1	0	0
ac	0	0	1	0	0	8	0	0	0	0	0
cc	10	3	36	0	0	3	9	0	0	19	0
fv	...	...	...	...	...	1,6	...	...	...	...	...
aa	0	0	0	0	12	2	0	0	1	0	0
ad	0	0	3	0	10	18	0	0	0	0	0
dd	0	15	33	0	1	22	5	31	55	9	7
fv	...	...	...	...	1,4	1,4	...	...	0.0	...	...
bb	11	1	13	64	16	95	13	3	37	0	93
bc	43	11	65	7	0	22	24	0	41	0	0
cc	10	3	36	0	0	3	9	0	0	19	0
fv	2,0	3,2	1,5	...	...	0,7	1,1	...	...	...	...
bb	11	1	13	64	16	95	13	3	37	0	93
bd	12	6	73	11	9	91	16	43	126	0	63
dd	0	15	33	0	1	22	5	31	55	9	7
fv	...	0,8	1,8	...	1,1	1,0	1,0	2,2	1,4	...	1,2
cc	10	3	36	0	0	3	9	0	0	19	0
cd	6	47	98	1	0	8	16	4	67	15	0
dd	0	15	33	0	1	22	5	31	55	9	7
fv	...	3,5	1,4	...	...	0,5	1,2	...	...	0,6	...

In the lower part of the table the absolut frequencies for calculating the factor f_v are written down.

EVALUATION OF PERCENTAGE OF SELFING IN SARDINIAN GERMPLASM OF *Lolium rigidum* Gaud. BY STARCH GEL ELECTROPHORESIS.

S. Bullitta , R. Floris , F. Veronesi

Centro Miglioramento Pascoli - CNR, Sassari, Italy
Istituto di Agronomia dell' Università, Sassari, Italy

Summary

Lolium rigidum Gaud. is an important annual self-reseeding forage grass in the Mediterranean areas. The percentage of selfing of a Sardinian population of this species has been detected by means of the PGI isozyme marker. Genotypes homozygous for different PGI-2 alleles have been crossed. The analysis of progenies showed a percentage of selfing equal to 7.5. As a consequence, breeding strategies typical of outbreeders must be applied to *L.rigidum*.
Keywords: *Lolium rigidum* Gaud., PGI, selfing percentage.

Introduction

Lolium rigidum Gaud. (2n=2x=14), is a widespread annual self-reseeding forage grass in pastureland areas of the Sardinian island (Bullitta, 1976). The "Nurra" population utilized in the trial was chosen among several germplasm accessions previously tested for agronomic characters. Aim of the research has been to study the percentage of selfing of the species in order to define a breeding program for the synthesis of a *L.rigidum* variety suitable for pastures improvement in Sardinia and in the Mediterranean areas.

Results and discussion

The presence of 5 alleles at the PGI-2 locus with frequencies as indicated on table 1 was detected in the "Nurra" population by means of starch gel electrophoresis, procedure according to Hayward & Mc Adam (1977). The population appeared to be in Hardy-Weinberg equilibrium (X^2 =20.21 n.s.).
In order to obtain information on the percentage of selfing, 550 seedlings obtained by controlled crossings among genotypes homozygous for different alleles at the PGI-2 locus were checked. The analysis showed a percentage of selfing equal to 7.5. Furthermore, the inbreeding depression on the number of culms, flag leaf area, plant height and dry matter yield was recorded comparing 50 F_1 and 50 S_1 plants grown under spaced plants conditions (40x40 cm) in open field. A strong inbreeding depression (table 2) appeared for the analyzed traits with the exception of plant height.

Table 1. Number of individuals in the phenotypic classes and frequencies (f) of alleles at the PGI-2 locus.

PGI-2	xx	1	f	x	0.0268
	xa	7		a	0.4048
	xb	2		b	0.0147
	xc	20		c	0.5475
	xd	0		d	0.0043
	aa	96			
	ab	5			
	ac	266			
	ad	0			
	bb	1			
	bc	8			
	bd	0			
	cc	167			
	cd	5			
	dd	0			

x= fastest; d= slowest

Table 2. Inbreeding depression in quantitative characters.

Characters	F_1 $\bar{x}$	S_1 $\bar{x}$	F	S_1/F_1
Number of culms	73.05	53.80	*	0.73
Average flag leaf area (cm^2)	2.54	1.62	*	0.63
Plant height (cm)	68.47	67	ns	0.97
Dry matter yield (g $plant^{-1}$)	42.48	24.12	**	0.56

** significant for $P \leq 0,01$, * $P \leq 0,05$; ns not significant

Conclusions

On the whole, the *Lolium rigidum* "Nurra" behaved as a typical outbreeding population with a low rate of selfing and a strong inbreeding depression; as a consequence, an intra-population breeding approach based on recurrent selection seems to be advisable for its improvement.

References

Bullitta, P., 1976. Un nuovo loglio annuale autoriseminante. L' Inf. agr. 36: 23945-23947.

Hayward, M & N. Mc Adam, 1977. Isozyme polymorphism as a measure of distinctiveness and stability in cultivars of *Lolium perenne*. Z.Pflanz. 79: 59-68.

DEVELOPMENT OF IN VITRO METHODS FOR FERTILIZATION STUDIES IN PERENNIAL RYEGRASS.

A. Elgersma and J. van Hateren

Centre for Plant Breeding Research CPO, PO Box 16, 6700 AA Wageningen, The Netherlands

Introduction

In perennial ryegrass seed yield is low and unreliable. Cultivars differ in seed yielding capacity (Elgersma, 1990). Environmental stress factors (e.g., low temperatures, drought) decrease seed yield. Pollination, fertilization and early stages of seed development are critical periods for the realization of the yield potential (Elgersma and Śnieżko, 1988). Stress tolerance during these periods is desirable for a high and stable seed yield. Genetic variation for stress tolerance can be exploited in a breeding program. Rapid and reproducible methods are needed for screening a large number of genotypes in various environments. Results obtained with these in vitro methods should correlate with results obtained in vivo. Some preliminary results are presented.

Materials and culture methods

Flowering plants were obtained after artificial vernalization, about 5 months after sowing. For controlled environment studies, plants are difficult to handle. In one ear many florets at different positions flower during one day. Some florets open earlier in the morning than others. For pollination studies, this is undesirable as each individual floret must be fixed after a defined period from its pollination. Excised ovaries put on agar in a Petri dish provide a useful system for pollen tube growth studies (Elgersma et al., 1989). The ovaries are all pollinated at the same moment and self-pollen is not present, contrary to the situation in ears and spikelets. Pollen germination and pollen tube growth can be screened with a UV-fluorescence microscope.

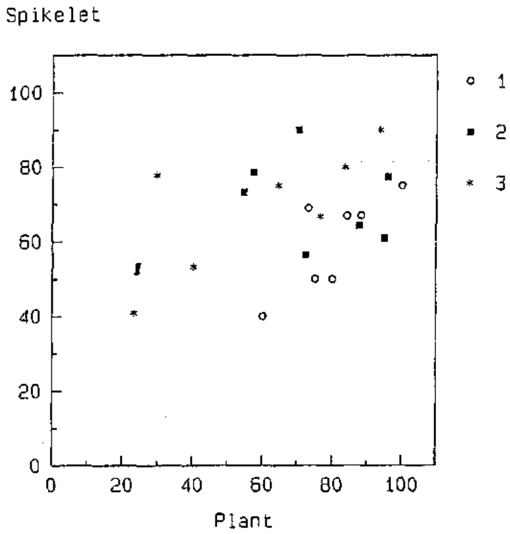
For seed development studies, detached spikelets were examined. In all spikelets the basal floret was about to flower. Sterilized spikelets were put on an aseptic agar medium containing sucrose (Trione and Stockwell, 1989). The spikelets flowered poorly because the relative humidity in the closed Petri dish was high. Pollination with pollen from sterilized spikelets was difficult. Much contamination occurred and only few, abnormal seeds were produced. However, non-sterilized spikelets put on water produced viable seeds. This simple and cheap system seems useful for studying fertilization and seed development.

Results and discussion

Viable seeds developed in detached spikelets and ears cultured on water. These systems can have practical applications, e.g. making crosses or selfpollinations, and can be used in fundamental research. Our aim was to use detached spikelets for fertilization studies. Results obtained in vitro and in vivo were compared.

Three experiments were conducted with 9, 8 and 8 genotypes from the cultivars Wendy, Talbot, Pablo and Lamora. Per genotype up to 10 spikelets were put on water and the florets were pollinated. Also on the intact plants florets were pollinated. Spikelets and plants were placed in a glasshouse at 17/12 °C and 16/8 h light. After one week seed set in the florets was assessed and the length of developed seeds was measured.

The correlation between seed set percentages measured in detached spikelets and in intact plants was positive, but low (Fig. 1). For seed length the relation between spikelets and plants was better (Fig. 2). This suggests that seeds elongate normally in detached spikelets. However, there were differences between the three experiments.



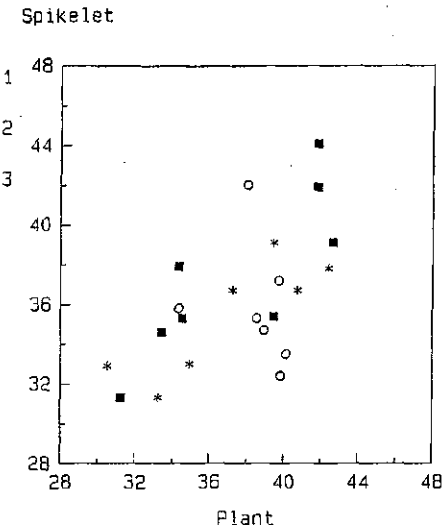


Fig. 1. Seed set (%) in detached spikelets and in plants in 3 experiments

Fig. 2. Length of seeds (ocular scale units) detached spikelets and in plants

Conclusions

Detached ovaries, spikelets and ears provide tools for studying pollen tube growth and seed development, respectively. However, for use as a screening system a strong similarity is required between in vitro and in vivo observations, which was not found. Non-sterilized ears and spikelets on water performed better than surface-sterilized materials on a medium containing sucrose. Viable seeds were harvested on detached spikelets and ears cultured on water. We are now optimising the culture conditions and studying the effect of temperature on seed set and development.

References

Trione, E.J. and V.O. Stockwell, 1989. Development of detached wheat spikelets in culture. Plant Cell, Tissue and Organ Culture 17: 161-170.

Elgersma, A. 1990. Genetic, cytological and physiological aspects of seed yield in perennial ryegrass (Lolium perenne L.). Doctoral Thesis, Agricultural University Wageningen, the Netherlands.

Elgersma, A. and R. Śnieżko. 1988. Cytology of seed development related to floret position in perennial ryegrass (Lolium perenne L.). Euphytica S: 59-68.

Elgersma, A., A.G. Stephenson and A.P.M. den Nijs, 1989. Effects of genotype and temperature on pollen tube growth in perennial ryegrass (Lolium perenne L.). Sex. Plant Reprod. 2:225-230.

COMPARISON OF CLOSELY RELATED DIPLOID AND TETRAPLOID FORMS OF ANNUAL RYEGRASS (Lolium multiflorum var. westerwoldicum)

U. FEUERSTEIN[1] and Chr. PAUL

Institute for Grassland and Forage Research, FAL, D-3300 Braunschweig-Voelkenrode, Fed. Rep. Germany

SUMMARY

A range of morphological characters, heading date and yielding ability were compared between newly induced tetraploids and their diploid progenitors. The results indicate that phenotypic expression in autotetraploids is not determined by chance but by the genotype of their diploid progenitors.
Keywords: grasses, annual ryegrass, polyploidy, morphological comparison, performance

INTRODUCTION

In forage plant breeding the induction of autotetraploids has always been of great practical significance. However, compared to the diploid (2x) level, the genetic variability in autotetraploid (4x) varieties is narrower.
In this situation it is difficult for the practical plant breeder to decide which resources should be allocated to the breeding programmes for autotetraploid and diploid varieties.

In order to characterize and evaluate the potential of polyploidy breeding in annual ryegrass a genetically broad range of 2x/4x forms was investigated. This aimed at comparing and testing:

- the mean expression of morphological and phenological characters in closely related 2x and 4x forms (Experiment I)
- the relationship between the expression of these characters at the 2x and 4x level (Experiment I)
- the agronomic performance of advanced 2x and 4x breeding lines and cultivars (Experiment II).

MATERIALS AND METHODS

Experiment I: In one location comparable pairs of 2x/4x genotypes derived from a broad range of annual ryegrass forms were formed on a single plant basis (225 diploid and tetraploid C_1-progenies resulting from 64 selfed mixoploid C_0-plants) with every pair being represented by three cloned replicates. A range of morphological characters and heading date as a phenological character was studied.

1 Pesent address: Deutsche Saatveredelung, Hof Steimke, D-2811 Asendorf, Fed. Rep. Germany

Experiment II: The agronomic performance of diploids (9 breeding lines and the cultivars Lirasand and Liwelo) and tetraploids (21 C_3-progenies (derived from the cultivar Lirasand) and the cultivars Aubade, Billion and Wesley) was tested at two locations in 5 m² plots by a lattice design (3 replicates). The sowing rate as 35 kg/ha for diploid and tetraploid material. The plots were harvested with a Haldrup plot harvester. For dry-matter (DM) evaluation the forage was dried for 24 hours at 105 °C.

RESULTS AND DISCUSSION

Tetraploids were markedly altered in morphology and phenological development when compared to their diploid progenitors. An increase in the size of vegetative and reproductive organs (except tiller length) was generally accompanied by a decrease in their number (except number of spikelets per ear).

Heading date on average was nearly 5 days later in the tetraploid plants than in the corresponding diploids.

The predictability of the phenotypic expression in tetraploid genotypes was quantified from the relationship between diploid and tetraploid progenies of mixoploid plants. As judged by r^2-values of the 4x/2x relationship the expression of certain plant characters at the tetraploid level could be predicted with some precision. Tetraploid predictability was highest for heading date. The difference in predictability for the various characters appeared to correspond to their differential heritability as observed in other studies.

Performance testing of advanced diploid and tetraploid material under break crop cultivation showed a differentiation in the relationship between yield and dry matter content. At given DM-contents the yield superiority of the tetraploids amounted to 12 % over the diploids. Assuming that this differentiation represents a ploidy effect, its utilisation for creating high yielding tetraploids on the basis of diploids combining high yield with a high DM-content is suggested.

The majority of the tetraploid breeding lines newly developed from the high yielding diploid cultivar Lirasand showed higher yields than their progenitor. They also surpassed the highest yielding tetraploid cultivar Aubade. This indicates that in addition to morphological and phenological characters, yielding ability can also be predicted when tetraploids are induced from diploids.

STATISTICAL ANALYSIS OF 2N AND 4N POLLEN FORMATION IN LOLIUM PERENNE USING POLLEN DIAMETERS

R.C. Jansen and A.P.M. den Nijs
Centre for Plant Breeding Research CPO,
P.O. Box 1600, 6700 AA Wageningen

It is a well-known feature that pollen size increases with ploidy level. Therefore, screening for genotypes that are able to produce 2n and 4n pollen can be based on differences in size of n, 2n and 4n pollen (Veronesi et al., 1988). A typical data set consists of measurements of pollen diameters presented in tables of observed frequencies or in histograms. Usually such a histogram shows an unimodal but skewed distribution of the data. One or more of these histograms together may suggest the presence of 2n or 4n pollen. A small data set, five samples from one and the same plant of Lolium perenne, is used to show the statistical analysis of such data (Fig. 1).

Mixtures of two, three and four normal densities were fitted to the logtransformed data. It appeared that the observed and estimated frequencies were very close for mixtures of three or four components (Table 1). The estimated mean values for the first three components correspond to pollen volumes in the ratio of 1 : 1.9 : 3.6. Therefore these components probably correspond to n, 2n and 4n pollen (Table 2 and Table 3). The fitted component densities as well as their mixture density are superimposed on the histograms (Fig. 1). The fourth component is solely due to the three smallest observations in the fifth sample, which represent probably letal micropollen.

The large variation in 2n and 4n pollen proportions between the five samples suggests a strong effect of environmental factors on 2n and 4n pollen production. Therefore, extreme caution should be taken in defining genotypes as 2n or 4n pollen producers.

Crude estimates of the proportions of 2n and 4n pollen can easily be obtained by the method proposed by Veronesi et al (1988). Therefore this method may be useful in breeding programs. The present method yields a more accurate analysis of the data by taking into account the pollen distribution (lognormal), the experimental design (samples), and the method of data collection (rounding). Inference on the number of components can be made using χ^2 tests for goodness of fit. The present method will be especially valuable for the analysis of experiments designed to study effects of factors (e.g. genotype, temperature, or age of the plant) on 2n and 4n pollen formation.

References

Veronesi F., Mariani A., Tavoletti S., 1988. Screening for 2n gamete producers in diploid species of genus Medicago. Genet. Agr. 42: 187-200.

Table 1. χ^2 test for goodness of fit of different numbers of components in the pollen mixture.

Number of components in the mixture	Degrees of freedom	χ^2 test statistic	Critical values (α = 0.05)
1	37	341.5	51.9
2	33	55.5	47.1
3	31	42.2	44.7
4	30	13.7	43.4

Table 2. Estimated proportions.

	Ploidy level		
Sample	n	2n	4n
1	0.91	0.09	0
2	1	0	0
3	1	0	0
4	0.12	0.69	0.19
5	0	0.98	0

Table 3. Estimated mean values.

	Ploidy level		
Sample	n	2n	4n
1	13.4	16.6	-
2	13.9	-	-
3	14.3	-	-
4	13.2	16.4	20.3
5	-	16.3	-

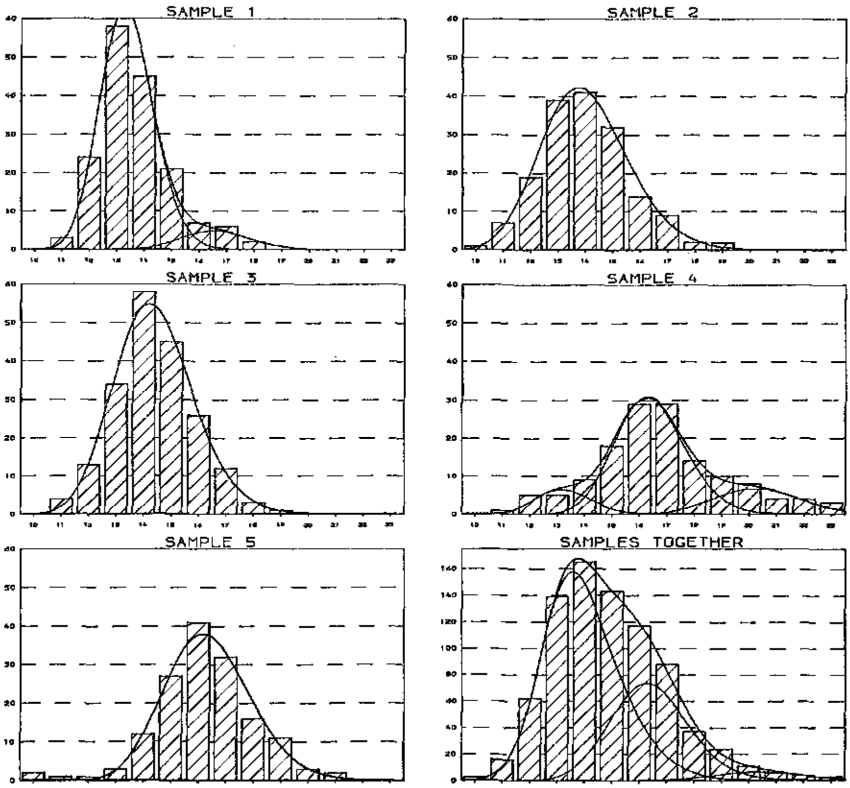


Figure 1. Histograms of the data and graphs of the n, 2n and 4n pollen densities and their pollen mixture density.

SPERM CELL ISOLATION FROM POLLEN OF PERENNIAL RYEGRASS (LOLIUM PERENNE L.)

H.M. VAN DER MAAS, F.A. KRENS & M.A.C.M. ZAAL

Centre for Plant Breeding Research CPO, Box 16, 6700 AA Wageningen

Summary

Perennial ryegrass (Lolium perenne L.) is important in Dutch agriculture, as meadow and fodder grass. For specific approaches in genetic manipulation, a sperm cell isolation procedure for perennial ryegrass has been developed. The yield of viable sperm cells varied from 10-15% in respect to the original amount of pollen grains. Cytological experiments confirmed that sperm cells of perennial ryegrass are true haploid protoplasts.
Keywords: perennial ryegrass, sperm cell, gametoplast, protoplast.

Introduction

Perennial ryegrass (Lolium perenne L.) is important in Dutch agriculture, as meadow and fodder grass. For further improvement of its agronomical traits, future breeding programmes might include cell and molecular biological methods. For specific approaches in genetic manipulation the gametes may provide an alternative to somatic cells. Therefore gametoplasts (protoplasts of gametes) should then be isolated. The pollen of perennial ryegrass is tricellular. This means that the mature pollen grain consists of a vegetative cell in which two sperm cells with their own plasma membrane are located. In order to isolate these sperm cells from the pollen grain an isolation procedure has been developed (Van der Maas & Zaal, 1990). Further optimization of this procedure and cytologic analysis of the sperm cells were performed.

Material & methods

Anthers just before anthesis were dissected from the spikelet. Subsequently, the anthers were squashed in a modified BKS medium (Brewbaker & Kwack, 1963). After filtering the suspension through a 20 m filter, the residue was squashed again in equal volumes of the modified BKS medium and a modified RY medium (Yamada et al., 1986). The suspension was filtered and the two filtrates were mixed. The modified RY medium was added to give a final BKS:RY ratio of 1:2. For further purification the resulting filtrate was layered onto a discontinuous Percoll gradient. After centrifugation the sperm cells were collected from the interphase. Subsequently, the purified sperm cell suspension was washed in modified RY medium and the yield was determined.

Results

Within 5 minutes after isolation, the shape of the sperm cells transformed from a spindle shape, which is the natural form within the pollen grain, into a spherical shape. To increase the amount of sperm cells that are released by an osmotic shock, the pH of the isolation medium was crucial. At pH 5.5, there were no osmotic effects of the isolation medium. However, when the pH was raised to pH 6.0, the yield was doubled because the isolation became dependent on the osmolality of the isolation medium. In the BKS medium with 10% sucrose the osmotic shock was optimal. To retain the viability of the sperm cells after isolation, foetal calf serum was essential. After varying the concentration of the calf serum, it was evident that at a concentration of 6.5% the sperm cells remained viable for a longer period when compared to lower concentrations of calf serum. Antioxidants were tested to optimize the viability and the yield of the sperm cells. The antioxidant vitamine C, added to the isolation media at a concentration of 10 mM, effected the viability of the sperm cells in a positive way. Because vitamin C often works as a synergist with vitamin E, the latter compound was tested in the presence of vitamin C. It was found that 0.1 mM vitamin E doubled the yield of viable sperm cells. After modifications of the former procedure (Van der Maas & Zaal, 1990), the yield of FCR positive sperm cells was raised from 5% to 10 - 15% in respect to the original amount of pollen grains. The viability period of the sperm cells was extended to 48 hours.

When sperm cells were stained for the presence of cellulose and callose with respectively calcofluor white and analine blue, no fluorescence was observed. This suggests that sperm cells of perennial ryegrass are true gametoplasts. To analyse the ploidy level of the isolated sperm cells, flow cytometric measurements (FCM) were performed. The fraction of sperm cells was shown to consist of haploid cells only.

Conclusions

With our isolation procedure for sperm cells of perennial ryegrass, viable male gametoplasts can be routinely obtained for future fusion and DNA transfer experiments.

References

Brewbaker, J.L., Kwack, B.H., (1963): The essential role of calcium ion in pollen germination and pollen tube growth. Am. Jour. Bot. 50:747-858.

Van der Maas, H.M., Zaal, M.A.C.M., (1989): Sperm cell isolation from pollen of perennial ryegrass (Lolium perenne L.). In: Barnabás, B., Liszt, K. (eds), Characterization of male transmission units in higher plants, p. 31-35.

Yamada, Y., Zhi-Qi, Y., Ding-Tai, T. (1986): Plant regeneration from protoplast-derived callus of rice (Oryza sativa L.). Plant Cell Reports 5:85-88.

A STRATEGY FOR PRODUCTION OF HAPLOIDS AND CHROMOSOME DOUBLED HAPLOIDS FROM ANTHER CULTURE OF PERENNIAL RYE-GRASS

S. Madsen, S.B. Andersen & A. Olesen

The Royal Veterinary & Agricultural University, Copenhagen, Denmark

Abstract

The ability to form haploid plants in anther culture of Lolium perenne is highly restricted to certain genotypes. Recently it has been shown that rare genotypes capable of producing 1-3 green plants per 100 cultured anthers can be found among commercial ryegrass varieties. Hybrid progenies from crosses between such responsive genotypes revealed recombinant types which produced 10-60 green plants per 100 anthers. Eleven such high responding "inducer" genotypes have been crossed to each of 10 clones of breeding material with high agronomic performance. The 120 offspring populations are being evaluated for the ability to respond in anther culture to clarify whether the trait can be transferred to ordinary breeding material of ryegrass through hybridization. The experiment is performed with two replications and approximately 60.000 anthers will be cultured. An estimate of the heritability of anther culture response is pursued together with estimates of general and specific combining ability. The results of the first replication are being analyzed while the second replication will be ready later. A recurrent selection scheme for utilization of high responding inducers in the breeding of ryegrass is proposed. The posibilities of constructing narrow synthetics and hybrid varieties using chromosome doubled haploids are discussed.
Keywords: Anther Culture, Haploids, Forage Grasses.

CYTOLOGICAL MECHANISMS RESPONSIBLE FOR 2N GAMETE PRODUCTION IN MEDICAGO SPP.

A. Mariani[1], S. Tavoletti[1] and F. Veronesi[2]

1 Centro di Studio per il Miglioramento Genetico delle Piante Foraggere, C.N.R., Perugia, Italy. 2 Istituto di Agronomia generale e Coltivazioni erbacee, Università degli Studi, Sassari, Italy.

Summary

A stain clearing technique has been used for the cytological analysis of microsporogenesis and macrosporogenesis of 2n gamete producer mutants. This methodology allowed the mechanisms of 2n gamete production to be identified and led to the finding that genotypes which produce 4n pollen are characterized by abnormalities during macrosporogenesis and female gametophyte development.
Keywords: meiotic mutants, 2n gametes, 4n gametes.

Introduction

Meiotic mutants which produce numerically unreduced gametes are particularly effective in ploidy manipulation and probably play a major role in the origin and evolution of polyploid series, due to their wide diffusion in the plant kindom. Gametes with the sporophytic chromosome number, 2n gametes, are the most common, but also the production of 4n gametes has been described in many plant species (Veilleux, 1985). In the *Medicago* genus the importance of 2n gametes has been stressed both in the evolution and breeding of cultivated alfalfa (*Medicago sativa* L.) (Bingham, 1980). The aim of this research was to identify the cytological mechanisms responsible for numerically unreduced gamete production in two previously selected clones, H33 producing 2n pollen and 2n eggs, and H25 producing 4n pollen and 2n eggs (Veronesi *et al.*, 1990).

Results and discussion

The stain clearing technique proposed by Stelly *et al.* (1984) was applied to study microsporogenesis, macrosporogenesis and female gametophyte development in clones H33 and H25. Moreover, pollen of the two mutants, together with normal pollen of diploid and tetraploid controls, was analyzed with a Scanning Electron Microscope (SEM) to verify differences which could be typical of meiotic mutants producing numerically unreduced gametes. The cytological analysis revealed that clone H33 produced 2n eggs of the SDR type (Second Division Restitution) and 2n pollen of the FDR type (First Division Restitution). In fact, in some ovules the II division cytokinesis was absent in the calazal dyad, and binucleated functional macrospores were produced; the fusion of the two nuclei gave rise to SDR 2n macrospores and unreduced embryo sacs. As regards microsporogenesis, normal tetrads of microspores where produced together with dyads of two 2n microspores and triads of one 2n and two n microspores due, respectively, to parallel and tripolar spindles at anaphase II. SEM analysis indicated the presence of differences in shape among grains, normal pollen being typically elliptical and unreduced pollen showing a higher size and irregular shape (Fig. 1A). As expected, normal diploid and tetraploid controls produced only elliptical pollen grains (Fig. 1B). The analysis of microsporogenesis of clone H25 indicated that the lack of cytokinesis was responsible for the production of monads instead of tetrads at the end of meiosis. Therefore only four-nucleated microspores were observed which evolved into 4n pollen grains with a polyhedric shape and strong stickiness (Fig. 1C). 4n pollen produced by H25 showed a low germination rate (28%), with some pollen grains (2%) growing 2 pollen tubes. As expected, no seeds were produced by crossing H25 as the pollen parent with one male sterile tetraploid clone and with normal diploids. In clone H25 also during macrosporogenesis the absence of cytokinesis was observed at the end of the I and/or the II meiotic division. As a consequence, bi- and trinucleated macrospores were produced; binucleated macrospores gave rise to 2n SDR embryo sacs. Moreover, aborted embryo sacs or embryo sacs with supernumerary polar nuclei were observed, probably produced by an abnormal development of the trinucleated macrospores.

On the whole, these results indicate that both the analyzed clones are SDR 2n egg producers, and clone H33 give rise also to FDR 2n pollen. Moreover, jumbo pollen production is confirmed to be associated with abnormalities during macrosporogenesis and female gametophyte development.

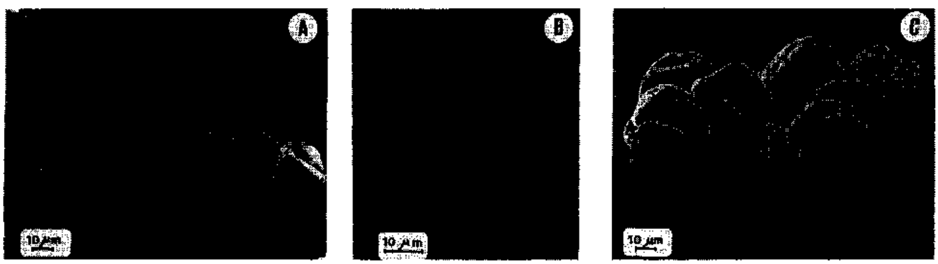


Fig. 1. A) Clone H33: n and 2n pollen. B) Normal pollen of a diploid control. C) Clone H25: 4n pollen.

References

Bingham, E.T., 1980. Maximizing heterozygosity in autopolyploids. In: W.H. Lewis (ed.). Polyploidy, biological relevance. Plenum Press. New York. pp. 471-489.

Stelly, D.M., S.J. Peloquin, R.G. Palmer & C.F. Crane, 1984. Mayer's hemalum - methyl salicylate: a stain clearing technique for observations within whole ovules. Stain Technol. 59: 155-161.

Veilleux, R., 1985. Diploid and polyploid gametes in crop plants. In: Janick J. (ed.): Plant Breeding Reviews. Vol. 3. AVI Publishing Company Inc, Westport, Connecticut. pp. 253-288.

Veronesi, F., S. Tavoletti & A. Mariani, 1990. Identification of 2n and 4n gamete producers in an experimental population of diploid Medicago. J. Genet. & Breed. 44: 143-148.

STUDIES TOWARD NOVEL METHODS IN BREEDING OF THE APOMICTIC SPECIES POA PRATENSIS L.

F. Matzk

Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, Germany

Summary

Three strategies in creating variability by recombination in pseudogamous Kentucky bluegrass (Poa pratensis L.) were proved: (1) induction of temporary sexuality by application of growth regulators, (2) in vitro regeneration of plants from immature endosperms, (3) use of plants without parthenogenetic capacity; only the last looks promising.

For selection of plants requiring fertilization of egg cells for embryo formation a fast and reliable new method was established. Inheritance of parthenogenesis was found to be dominant over obligatory fertilization. The selected sexual plants lacked all genes/alleles for parthenogenesis while the apomictic varieties were heterozygous. Crosses of sexual individuals with various apomictic varieties resulted in sexual as well as higly apomictic F_1 hybrids. A breeding programme with recurrent hybridization can be recommended.

Keywords: Poa pratensis, apomixis, inheritance of parthenogenesis, endosperm culture, growth regulators.

Introduction

Most of the present varieties of the apomictic Kentucky bluegrass (Poa pratensis L.) originated as individual plant selections collected from old turf areas or grasslands. Further successful breeding requires new strategies to create new variation.

Juleen (1960) and Grazi et al. (1961) observed an increased degree of sexuality after X-irradiation in the cv. Fylking. Pepin and Funk (1971) successfully used a method of intraspecific hybridization with the cv. Bellevue. Some other breeders, however, could not reproduce these results with their breeding stocks. As an alternative to recombination via sexual processes, Wu and Liu (1985) as well as Nijs (1990) studied somaclonal variation in Poa pratensis; but the variability found is too low for using in breeding. To overcome these unsatisfactory situation, three other strategies were studied and results are described in this paper.

Results and discussion

Application of different growth regulators (synthetic auxins, zeatin, gibberellin, oxopyridazine) at certain concentrations and developmental stages (between meiosis and anthesis) did not result in enhanced sexuality.

In vitro regeneration of plants from immature endosperms was tested as a further potential source for variability since in pseudogamous Kentucky bluegrass the endosperm is a product of fertilization. Endosperm explants without embryo and hulls at 14 to 27 days after pollination were inoculated on 7 differently modified MS-media. Callusses and hairy roots could been induced from endosperm at milk-waxy-ripeness, but plants did not regenerate.

Genetically controlled sexuality could successfully be used to set free variability for bluegrass breeding. However, classification in sexual or apomictic plants was difficult till now, because the procedures usually used could not distinguish between offtypes resulting from hybridization or from other sources of variability ("haploid" parthenogenesis, chimerism, or somatic chromosomal instability). This handicap has been overcome by a recently developed method for rapid and efficient identification of plants that are incapable of parthenogenesis (Matzk, 1990).

Results with five selected clones without parthenogenetic capacity indicate genetic control of parthenogenesis. None of the 70 progeny plants from self-pollination as well as from crosses with other sexual plants showed parthenogenesis. After open pollination or pollination of emasculated panicles with pollen from apomictic varieties hybrids without (77) and with high (25) parthenogenetic capacity arose besides plants with a moderate degree of parthenogenesis (34). Such results may be expected if the mother plants lack alleles for parthenogenesis (nulliplex) while the polyploid pollinators should be heterozygous with one or more dominant alleles. In addition, dosage effects and/or modifying genes are probably included.

Results of F_2 and S_2 segregations have confirmed the above assumptions of genetic control. The offsprings of hybrids with high parthenogenetic capacity were stable apomicts. Very strong inbreeding depression was observed in progenies of the self-pollinated sexual clones. The results indicate that recurrent hybridization may be useful for improvement of Kentucky bluegrass.

References

Grazi, F., M. Umaerus & E. Akerberg, 1961. Observations on the mode of reproduction and the embryology of Poa pratensis. Hereditas47: 489-541.

Julen, G., 1960. The effect of X-raying on the apomixis in Poa pratensis. Genet. Agrar. 13: 60-66.

Matzk, F., 1990. A novel approach to differentiated embryos in the absence of endosperm. Sex. Plant Reprod. 3: in press.

Nijs, den A.P.M., 1990. Experimenting with apomixis and sexuality in Poa pratensis. Apomixis Newsl. 2: in press.

Pepin, G.W. & C.R. Funk, 1971. Intraspecific hybridization as a method of breeding Kentucky bluegrass (Poa pratensis L.) for turf. Crop Sci. 11: 445-448.

Wu, L. & L. Liu, 1985. Callus culture and genetic constancy among regenerated plants of Kentucky bluegrass (Poa pratensis L.) cultivars. Proc. 5th Int. Turfgrass Res. Conf., Avignon, p. 175-183.

COMPARISON BETWEEN 2X AND 4X ISOGENIC CLONES OF RED CLOVER

C. Mousset-Déclas and A. Jam

Institut National de la Recherche Agronomique, Dijon, France

Summary

Four diploid and tetraploid isogenic clones of red clover (*Trifolium pratense* L.) were compared. The expression of the leucine-aminopeptidase isozymes remains identical in tetraploid compared to diploid. The comparison of morphologic characters on the plants, the inflorescences and the leaflets indicates significative increases in the tetraploid plants for the thickness of stem, the length of the corolla, the length and width of the corolla tube, the surface, length and width of the leaflet. However the number of stem and the number of flower per head are unchanged. The forage quality analyses indicates an important decrease of the dry matter content in tetraploid plants and an increase of the digestibility. The variation of the watersoluble carbohydrate content and of the protein content depends of the family.
Keywords : red clover, Trifolium pratense, polyploidy, tetraploidy.

Introduction

To improve tetraploid cultivars of red clover it is interesting to better understand the action of the polyploidisation on morphologic, physiologic and agronomic characters. Few studies were based on real isogenic 2x-4x clones but more on diploid and tetraploid varieties and families, so with different genotypes. This study presents the first results on comparison of 4 isogenic 2x-4x clones of red clover.

Material

The 4 isogenic clones were obtained from 3 families of the french diploid cultivar DIPER by an in vitro chromosome doubling method using colchicine (Mousset-Déclas et al., 1989). Each diploid and tetraploid plant was submitted to the same colchicine treatment.

Result and discussion

Isozyme electrophoresis

The leucine-aminopeptidase (LAP) system was used to compare the diploid and tetraploid clones. A polyacrylamide gel electrophoresis was realised with limb fractions. No differences were observed between 2x and 4x forms of each family but differences were found between the 4 families.

Morphologic comparison

On each plant, 8 morphologic characters were measured by hand :

- number of flowering stems,
- thickness of 5 flowering stems,
- number of inflorescences,
- number of multiparted inflorescence,
- number of flowers per head (for 3 heads per plant),
- length of corolla) for 10 flowers
- length of corolla tube) per head and
- width of corolla tube) 3 heads per plant

Using an image analysis system, 20 leaves (60 leaflets) per clone were mesured for perimeter, surface, length, width and length/width of each leaflet.

Table 1. Variance analyse of 13 morphologic characters mesured on 4 isogenic 2x-4x clones (°mesured in centimeter, °°probability of F test, NS = no significant).

	Effect of ploidy		
	2x	4x	P°°
-Number of flowering stems	13.16	12.08	NS
-Thickness of flowering stems	3.6	4.7	<0.01
-Number of inflorescence	34.94	25.32	<0.05
-Number of multiparted inflorescences	1.78	4.61	<0.01
-Number of flowers/head	93.81	95.80	NS
-Length of corolla°	1.59	1.71	<0.01
-Length of corolla tube°	0.92	0.98	<0.01
-Width of corolla tube°	0.21	0.23	<0.01
-Perimetre of leaflet°	10.58	12.58	<0.01
-Surface of leaflet°	28.20	39.53	<0.01
-Length of leaflet°	4.03	4.68	<0.01
-Width of leaflet°	2.30	2.83	<0.01
-Length/width of leaflet	0.043	0.041	<0.01

Each character is significatively different in tetraploid compared to diploid forms, except for the number of flowering stems and the number of flowers per head (table 1). All the morphologic traits mesured increase in tetraploid forms apart the number of inflorescences and the length/width ratio for the leaflet.

Forage quality

At the prebloom stage each plant was cut (except for the familie FY1 : only the leaves were cut). The forage was weighted, dried at 60°C then ground to 0.8 mm and analysed for protein content, watersoluble carbohydrates content and in vitro digestibility. The dry matter is significantly lower in tetraploid compared to diploid forms, but the digestibility is higher. The protein content and the watersoluble carbohydrates fluctuate with the family and more replications are necessary.

Conclusion

For morphological characters and forage quality, tetraploid plants appear different compared to isogenic diploid plants. Others studies based on diploid and tetraploid families and varieties have been obtained the same results except for the number of stems, inflorescences and flowers (Paatela, 1962 ; De Roo, 1975). In our experiment, the tetraploid plants analysed are the C0 generation (directly from the colchicine treatment). No inbreeding effect is observed in opposition with Gillet & Gallais (1985) experiments.

References

De Roo, R., 1975. Recherches morphologiques comparatives sur les plantes autotétraploïdes à partir de leurs formes diploïdes chez quelques graminées et trèfles. IV Le trèfle violet (Trifolium pratense L.). Rev. de l'Agriculture belge 28:69-98.

Gillet, M. & A. Gallais, 1985. Can "doubling depression" throw some light on the heterosis process ? Agronomie 5(7):665-666.

Mousset-Déclas, C., J.A. Anderson, E.G. Williams & N.L. Taylor, 1989. An *in vitro* chromosome doubling method for interspecific hybrids of *Trifolium*. XVI Int. Grassl. Cong., Nice, France, AFPF. p. 423-424.

Paatela, J., 1962. Characteristics of some diploid and tetraploid varieties of the late clover Trifolium pratense v. subnudum subv. serotinum. Acta. Agral. Fenn. 99:4-31.

NON-RANDOM MATING IN POLYCROSSES OF <u>FESTUCA</u> <u>PRATENSIS</u> IN DIFFERENT ENVIRONMENTS

Astrid Myhre and Odd Arne Rognli
Department of Biotechnological Sciences, Section Genetics and Plant Breeding
Agricultural University of Norway, Ås, Norway

Introduction

Polycrosses are widely used for progeny testing in breeding programmes. In Norway the systematic latin-square design suggested by Olesen and Olesen (1973) has been preferred. One of the key assumption underlying the use of polycross is that male gametes have to be incorporated equally and at random over the array of maternal genotypes. If not, the half-sib family structure the effective population size and the effective male sample size will be changed from expected, resulting in biased estimates of breeding values.

The parental genotypes in the polycross and their half-sib families have been analyzed for polymorphic isozyme loci in order to study the randomness of mating between the parental genotypes. By comparing the genetic constitution of the same half-sib families produced in different locations, possible environmental effects have been enlightened.

Materials and methods

The parental genotypes in the polycross are selected from a population from Svanhovd in the notheastern part of Norway (69 25'N). The population was an old ley originally sown with the variety 'Løken'. 22 genotypes with the best general performance were transferred to Ås outside Oslo (59 40'N) and to Tromsø (69 40'N), where they were planted in the same polycross design.

100 plants were randomly selected from each half-sib familiy of the 2 polycrosses and analyzed for the 3 polymorphic isozyme loci *Gpi1*, *Gpi2* and *Sod1*.

Results

The isozyme data show that non-random mating has occurred in the polycross at both locations.

The deviations from expected allele frequencies are highest for the alleles at the *Gpi2* locus. Unique allele combinations of two of the parental genotypes (*Gpi2-ab* and *Gpi2-bd*) made it possible to decide to which degree plants of these genotypes had contributed to the mating of the other parental genotypes in the two polycrosses.

When planted out at Ås in Southern Norway, the observed frequencies of the *Gpi2-a* allele in all 22 half-sib families were up to about one thousand times higher than expected from random mating. In Tromsø however, the *Gpi2-d* allele occurred at much higher frequencies than expected in all half-sib families.

Discussion

The observed non-random mating and inequal contribution from the parental genotypes can be explained by their different performance in the southern and northern locality. At Ås, the parental genotype containing the *Gpi2-a* allele showed superiour seed yield and the highest dry matter production throughout two years compared to the other parental genotypes (Aastveit and Aastveit 1990). This genotype did also have panicle emergence dates at the mean of all parental genotypes when grown in Southern Norway. We do not have corresponding data from the performance of the parental genotypes grown at the Tromsø location.

However, results from artificial selection studies and freezing experiments with isozyme-selected meadow fescue show an association between frost tolerance following a specific hardening procedure and the *Gpi2-d* allele in this plant material (Myhre, publ.1991). The parental genotypes in the polycrosses are planted out the year before the flowering takes place. An indirect selection through winter hardiness of the parental genotypes is therefore possible on all stages between the pollen/ovule formation and viable embryo formation.

Conclusions

The observed non-random mating gives reduced effective population size and changed half-sib family structure as compared to random expectation. The reduced resulting genetic variation between the half-sib families limit the value of polycross as a progeny testing method.

Natural selection in the formation of the half-sib families can be strong and create considerable deviations when the same polycross is grown in different environments. The location for growing polycross fields should therefore be chosen considering the region where the breeding material is to be used.

References

Aastveit, A.H. and Aastveit, K. 1990. Theory and application of open-pollination and polycross in forage grass breeding. Theor. Appl. Genet. 79:618-624.

Olesen, K. and Olesen, O.J. 1973. A polycross pattern formula. Euphytica 22:500-502.

IDENTIFICATION OF WHITE CLOVER POPULATIONS BY POLYACRILAMIDE GEL ELECTROPHORESIS OF SEED PROTEINS

M. Odoardi, L. Valdicelli, E. Piano

Istituto Sperimentale per le Colture Foraggere, Lodi, Italy

Summary

The banding pattern of seed storage proteins resulting from Polyacrylamide Gel Electrophoresis has been used to distinguish between cultivars and natural populations of Trifolium repens L. Suitable conditions of protein extraction and gel running allowed a clear differentiation of the subspecies hollandicum and giganteum. As for other forage species, seed protein banding pattern has potential as a tool for taxonomic classification.
Keywords: Trifolium repens, classification, electrophoresis.

Introduction

White clover (Trifolium repens L.) is an important pasture legume, widely diffused all over the world, and of special interest in EEC countries and in the irrigated areas of the Po valley, where the Ladino types originated. At the Experimental Institute of Fodder Crops, in Lodi, studies on white clover seed production have been carried out since the 1950's (Paoletti and Locatelli, 1989). A breeding programme is now in progress, starting from the preliminary evaluation of local populations as well as commercial ecotypes and cultivars (Annicchiarico, 1990). A biochemical study of the distinctness of such material is of great importance to allow a rapid discrimination and characterization of the germplasm under study, in addition to the differentiation for agronomic and morphological characters, usually strongly affected by the environment. As for many other crops, electrophoretic methods could effectively be used to discriminate among cultivars of white clover: the present paper describes the differentiation acquired with the electrophoretic procedure.

Results and discussion

A discontinuous Laemmly-type method defined by the International Seed Testing Association (ISTA) for a collaborative testing of pea cultivar identification (Cooke, 1989) has been applied to white clover seeds with minor modifications. As far as 30 different varieties and commercial ecotypes of various origin, listed in table 1, have been analyzed by Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). This technique allows the separation of the different sized proteins in the total seed populations, giving a characteristic banding profile highly reproducible also in the case of outbreeding species like white clover. In the present work, ground bulk seed meal has been extracted at least twice, giving a reproducible protein pattern; in 12 cases, different seed lots of the same cultivar were available and the respective electrophoregrams were identical, confirming that seed protein patterns are specific and unaffected by growing conditions.

SDS-PAGE electrophoregrams contained a large number of bands, over 15, in the range of molecular weight 90-14 kiloDaltons. An interesting variation has been shown by a single band (MW = 66 kD), always present in the cvs of the ssp giganteum, while not detectable, with two exceptions, in the hollandicum group of varieties. The cultivar S 184, reported as T. repens ssp sylvestre, more closely related to hollandicum types, lacks the characteristic band 66 kD too; on the contrary Irrigation and Merwi, described as ssp hollandicum, clearly show in their pattern the polypeptide 66 kD. The presence of giganteum germplasm in their genetic background can be inferred, but the hypothesis requires further evidences. A third group of varieties can be identified on the basis of the faint staining of band 66 kD: they belongs to the ssp giganteum and show additional characteristics (cyanogenesis, leaf size) typical of the "Group III Large" according to the classification of Caradus et al. 1989.

Table 1. List of varieties and commercial ecotypes (*) tested, with their country of origin, the subspecies and the banding pattern: band 66kD present (+), absent (-), weakly stained (+-).

Cultivar	Origin	Ssp	Band 66kD	Cultivar	Origin	Ssp	Band 66kD
ARAN	IRL	GIG	+-	MERWI	B	HOL	+
BARBIAN	NL	HOL	-	MILKA	DK	HOL	-
BLANCA RvP	B	HOL	-	MILKANOVA	DK	HOL	-
CALIFORNIA	USA	GIG	+	NFG GIGANT	D	GIG	+-
CANOPY	USA	GIG	+	OLWEN	UK	GIG	+-
CRAU	F	GIG	+-	REGAL	USA	GIG	+
CROWN	USA	GIG	+	REMA	PL	GIG	+-
ESPANSO	I	GIG	+	ROSS	IRL	GIG	+-
G. HUIA	NZ	HOL	-	SACRAMENTO	USA	GIG	+
G. KOPU	NZ	GIG	+-	S 184	UK	SYL	-
G. PITAU	NZ	HOL	-	SEM. OSCEOLA	USA	GIG	+
IRRIGATION	AUS	HOL	+	TITAN	USA	GIG	+
LADINO G. LOD.	I	GIG	+-	*CAVOUR	I	GIG	+
LIREPA	D	HOL	-	*CREDERA	I	GIG	+
LUNE DE MAI	F	GIG	+-	*QUARTESOLO	I	GIG	+

The whole variation of the protein pattern in SDS-PAGE was remarkable. The identification of single populations by the electrophoregrams has been possible when the patterns were carefully evaluated also in the lower region of the gels.

References

Annicchiarico, P., 1990. Collecting Ladino white clover ecotypes in the Po valley. Plant Gen. Res. Newsl. (in press).

Caradus, J.R., A.C. MacKay, D.R. Woodfield, J. van den Bosch, S. Wewala, 1989. Classification of a world collection of white clover cultivars. Euphytica 42: 183-196.

Cooke, R.J., 1989. The use of electrophoresis for the distinctness testing of varieties of autogamous species. Plant Var. & Seeds 2:3-13.

Paoletti, R., C. Locatelli, 1989. Seed yield, fodder yield and persistence in white clover cvs of different origins. Proc. XVI Int. Grassland Congress, Nice, France. p. 655-656.

ANDROGENETIC RESPONSE IN LOLIUM PERENNE: 90 GENOTYPES SCREENED, COLD PRETREATMENT, 5 MEDIA

H-G. Opsahl-Ferstad

Dept. Biotechnological Sciences, Agricultural University of Norway, Box 40, N-1432 ÅS-NLH, Norway

Summary

Ninety naturally adapted Norwegian genotypes of *Lolium perenne* were screened for their response to anther culture. 75 genotypes gave embryoids, 65 regenerated plants but only 15 regenerated green plants. Albinism is the big problem concerning the tecnique; 97% of the regenerated plants were albino. Cold pretreatment (4°C) and 5 media (LS, LS-Bante, 190-2, potato II (pII), pII with charcoal) were tested on selected genotypes. The effect of cold pretreatment differs between genotypes. The media 190-2, pII and pII in double layer, with charcoal, produces high amounts of embryoids. The regeneration is beeing registrated at the moment.
Keywords: androgenetic response, *Lolium perenne*, cold pretreatment, media.

Introduction

Plant breeders are interested in using dihaploid (DH) grasses to breed better controlled synthetic varieties or F1-hybrids. Naturally adapted Norwegian genotypes of *Lolium perenne* were screened for their response to anther culture. Further cold pretreatment and different media were tested on 9 and 4 selected genotypes, respectively. This because the technique needs to work better before it can be used efficient in breeding programmes.

Material and methods

Ninety naturally adapted Norwegian genotypes of *Lolium perenne* from 9 populations were used as well as 3 Danish highly responsive genotypes (175, 245 and 255) as references. 35 000 anthers were plated. Cold pretreatment (4°C) for 2 weeks were tested, compared to no pretreatment (Bante et al. 1990). 36 000 anthers were plated. Further five induction media were tested; pII medium (Wang & Hu 1984), pII medium, double layer with charcoal (Johansson 1986), LS- medium (Lindsmayer & Skoog 1965), LS-Bante medium (Bante et al. 1990) and 190-2 medium (Wang & Hu 1984). 20 000 anthers were plated.

Results and discussion

Since the regeneration is beeing registrated at the moment, the discussion concerning experiment 2 and 3 is preliminary. It will be presented at a poster at the meeting. In experiment 2 and 3, there were 2 "non-responsive" genotypes from experiment 1, that both responded in these experiments. That might indicate that all genotypes are able to respond to anther culture.

Screening, experiment 1

Approx. 8 500 embryoids, 3 000 albino plants and 90 green plants were obtained. Albinism is a big problem; 97% of the plants regenerated were albinos. 80% of the green plants regenerated were diploid (2X=14). Isozyme analysis using starch gel and the enzymes PGI-1, PGI-2 and GOT-2 were done. We identified heterozygote plants and plants having other bands than the donorplants using PGI-2. The analysis of variance showed there were significant differences between genotypes, concerning the response, but not between populations. The field material, one replication, was very difficult to sterilize and was damaged by thrips. This lowered the mean values. The most embryogenic genotype, 9-5, gave more then 300 embryoids per 100 anthers, while the best green plant producer, 7-5, regenerated 8 green plants per 100 anthers. The best Danish genotype gave 50 embryoids and 3 green plants respectively.

Pretreatment, experiment 2

Two weeks cold pretreatment influenced the genotypes differently. It increased the embryogenesis on genotype 9-5 (highly embryogenic), had hardly any effect on 7-5 (best producers of green plants) but decresed it in 245 (highly resposive Danish genotype) (Olesen 1987).

Media, experiment 3

The media seem to influence the genotypes more consistent. The media 190-2, pII and pII in double layer, with charcoal in the bottom one, gave many embryoids. LS and LS-Bante gave no and hardly any embryoids, respectively. 190-2 and pII-charcoal gave the same amounts of embryoids over genotypes (1 500, first replication), while pII gave some less (900). 7-5 (best green producer) gave 3 times as many embryoids on the pII-charcoal, while 9-5 (most embryogenic) prefered 190-2. The non-responsive genotype (1-1) gave embryoids on the pII-charcoal. It also seems like the pII-charcoal gave embryoids sooner than the other media.

References

Bante, I., T. Sonke, R.F. Tandler, A.M.R. van den Bruel, E.M. Meijer, 1990. Anther culture of Lolium perenne and Lolium multiflorum. Acta Bot. Neerl. 39(1):103.

Johansson, L., 1986. Improved methodes for induction of embryogenesis in antherculturs of Solanum tuberosum. Potato Research 29:179-190.

Linsmaier, E. & Skoog, F., 1965. Organic growth factor requirements of tobacco tissue cultures. Phys. Plant. 18:100-127.

Olesen, A., 1987. PhD-thesis in plant breeding. The Royal Veterinary and Agricultural University of Denmark, 209p.

Wang, X. & Hu, H. 1984. Plant Sci 36:237-239.

USEFULNESS OF CONFIDENCE INTERVALS FOR GENETIC PARAMETERS TO CHOOSE A METHOD OF SELECTION

C. RAVEL, G. CHARMET

INRA (National Institute of Agronomical Research) CLERMONT-FERRAND - FRANCE

SUMMARY

Confidence intervals for heritability and expected genetic advance of seven traits in two populations of perennial ryegrass (LOLIUM PERENNE) have been computed by two ways (bootstrap and parametric methods) for different combinations of selection. Two conclusions can be drawn from our results :
- superiority of individual selection
- usefulness of methods based on resampling to estimate confidence intervals for genetic parameters.
Key words : heritability, expected genetic advance, LOLIUM PERENNE, mass selection, family selection.

INTRODUCTION

A population structured in half sib families can be improved by several strategies : the (between) family selection, the individual (mass) selection, and the combinations of selection among Half-Sib families. The optimum strategy (maximises the expected genetic advance) depends on genetic parameters. The determination of the most efficient breeding method needs knowledge of the true genetic parameters of the population to be improved.

Two populations of perennial ryegrass built by one generation of random mating have been studied in order to find the most efficient breeding method.

MATERIAL AND METHODS

Two populations of perennial ryegrass structured in half sib families (population A : 49 progenies, population B : 43 progenies) have been put into trial during two years as spaced plants in a two randomized blocks design nursery with 15 plants per family and per block.

Seven traits (alternativity, rust resistance, spring growth, early and late aftermath heading, summer and autumn growth) have been scored on a 1 to 9 scale (1 : very good, 9 : very bad). The SMITH-HAZEL's index has been computed with the marks. Let G and P be the genotypic and the phenotypic variance-covariance matrix, a and b to be respectively the vector of economic weight (a : 1 ; 2 ; 2 ; 0.5 ; 0.5 ; 2 ; 2) and the vector of index coefficients, then $b = P^{-1}. G. a$.

According to the king of selection, G and P are different. The vectors of expected genetic gain is given by g = G. b. i/ i (i : selection intensity, i : the standard deviation of the index).

For each population, five combinations of selection pressure giving rise to the same final selection intensity are compared ((1) : mass selection - (2) : 50 % among family and 10 % within family - (3) 22,3 % among and 22,3 % within - (4) 10 % among and 50 % within - (5) family selection).

Confidence intervals for heritability and expected genetic advance have been computed by two ways (parametric method and a resampling method).

RESULTS - DISCUSSION

Resistance to rust, aftermath heading are heritable (0.5 h 0.7) and, thus, easy to improve. In contrast, spring, summer and autumn growth are difficult to improve (0.1 h 0.4). A quite high correlation between visual yield scores and green forage weight (TORRIE, 1957), and the low heritability of the production characters (VAN BOGAERT, 1977) explain that yield is difficult to improve.

Parametric and bootstrap methods give approximatively the same confidence intervals.

Whatever the method used to compute confidence intervals, they are very close. Mass selection is the most efficient method. (Mass selection gives the best maximum expected response).

Genetic parameters are helpful for plants breeders. Another outcome for them is the superiority of individual selection. But family selection seems to be useful for improving yield.

Comparing the confidence intervals obtained by the parametric formulae or by the resampling bootstrap method is interesting for a biometrician.

REFERENCE

BOGAERT G. van, 1977 : Scope for improving the yield of grasses and legumes by breeding and selection. In : Gilseman B. (ed) Proc. Int. Meeting on Animal Production from Temperate Grassland. An Foras Taluntais, Dublin, pp 29-35.

TAI G.C.C., 1986 : *A method to construct confidence intervals for expected* response trait selection. Theor. Appl. Genet. 71 : 595-599.

TORRIE J.H., 1957 : Evaluation of general and specific combining ability in perennial ryegrass (LOLIUM PERENNE L.). N.Z. Journal of science and technology 1025-1035.

THE POSSIBILITY OF USING GAMETOPHYTIC SELECTION IN BREEDING LUCERNE (MEDICAGO SATIVA L.)[1]

D. Rosellini*, F. Veronesi**, M. Falcinelli*** and F. Lorenzetti***

* Istituto Sperimentale per le Colture Industriali, Ministero dell'Agricoltura e delle Foreste, Bologna, Italy
** Istituto di Agronomia generale e Coltivazioni erbacee, Università degli Studi di Sassari, Italy
*** Istituto di Miglioramento Genetico Vegetale, Università degli Studi di Perugia, Italy

Summary

The chance of a lucerne ovule being fertilized is inversely related to its distance from the stigma. Therefore, selection of basal seeds in mature legumes may increase microgametophytic vigor; if considerable overlap exists, such selection may influence the vigor of the sporophyte. Two cycles of selection of apical (A) and basal (B) seeds did not effect dry matter yield and plant height, but seed yield and 1000 seed weight of B plants were increased with respect to A plants.
Keywords: gametophytic selection, seed yield.

Introduction

Selection for high pollen tube competitive ability during fertilization often determined correlated responses at the sporophytic level for important agronomic traits (Ottaviano & Mulcahy, 1986).

On the basis of the reproductive biology of lucerne (Medicago sativa L., 2n=4x=32), it may be assumed that it is possible to apply a selective pressure for microgametophytic growth vigor. In fact, the chance of a lucerne ovule being fertilized is inversely related to its distance from the stigma (Cooper et al., 1937; Barnes & Cleveland, 1963). If basal ovules are fertilized by more competitive microgametophytes, selection of basal seeds in mature pods may increase microgametophytic vigor; if it is at least partly controlled by genes also expressed in the sporophytic phase, such selection may influence the vigor of the sporophyte and its productive performance. On the contrary, selection of apical seeds would keep selective pressure for microgametophytic vigor to a minimum.

The aim of the present research has been to assess the effect of the selection of basal and apical seeds on sporophytic vigor in lucerne.

Materials and methods

One thousand pods with at least 8 seeds per pod were chosen (ten per plant) from 100 plants of the cv. "Adriana". From each pod, the apical

1. Research supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Italy, Research Project of National Interest "Biology and Tecnology of seed production".
An extended version of this paper will be submitted for publication elsewhere.

seed and the basal seed were picked up, thus obtaining two seed lots: A=apical and B=basal. A second cycle of microgametophytic selection was applied collecting apical seeds from A plants and basal seeds from B plants. Two hundred and fifty A plants and 250 B plants derived from the second cycle of selection were evaluated for agronomic traits in the field.

Results and Discussion

Dry matter yield at the first cut and at seed harvest and plant height of A and B plants did not significantly differ (table 1). On the contrary, seed yield and 1000 seed weight of B plants were significantly higher than those of A plants.

Variability of seed yield was also affected by selection, which determined a lower coefficient of variability for B plants (0.55) than for A plants (0.74); similar results were obtained in other species (Ter-Avanesian, 1978).

Further cycles of selection will be conducted to assess the potentiality of this gametophytic selection technique in breeding for quantitative traits in lucerne.

Table 1. Agronomic traits of A and B plants.

Agronomic traits	---- A plants ----- obs. no.	mean (1)	C.V.	---- B plants ----- obs. no.	mean (1)	C.V.
D. M. yield, 1st cut, g./plant	228	11.62 a	0.61	221	11.62 a	0.60
D. M. yield, seed harvest, g./plant	216	73.03 a	0.52	213	76.81 a	0.50
Plant height, cm	216	80.50 a	0.18	216	82.50 a	0.16
Seed yield, g./plant	216	16.03 a	0.74	213	18.37 b	0.55
1000 seed weight, g.	100	2.05 a	0.13	100	2.13 b	0.13

(1) Means on the same line followed by different letters are significantly different at 0.05 level according to Duncan test.

References

Barnes, D.K. & R.W. Cleveland, 1963. Genetic evidence for non-random fertilization in alfalfa as influenced by differential pollen tube growth. Crop Science 3: 295-297.

Cooper, D.C., R.A. Brinks & H.R. Albrecht, 1937. Embryo mortality in relation to seed formation in alfalfa (Medicago sativa L.). American Journal of Botany 24: 203-213.

Ottaviano, E. & D.L. Mulcahy, 1986. Gametophytic selection as a factor of crop plant evolution. In: Barigozzi, C.: The origin and domestication of cultivated plants. Developments in agricultural and managed-forest ecology. Elsevier, Amsterdam, Oxford, New York, Tokyo, 19: 101-119.

Ter-Avanesian, D.V., 1978. The effect of varying the number of pollen grains used in fertilization. Theoretical and Applied Genetics 92: 77-79.

REPRODUCABLE REGENERATION OF CALLUS CULTURES DERIVED FROM MATURE EMBRYOS IN LOLIUM PERENNE

Schmidt, F.[1] and Posselt, U. K.[2]

[1] Forschungsschwerpunkt Biotechnologie und Pflanzenzüchtung
[2] Landessaatzuchtanstalt, Universität Hohenheim
Postfach 70 05 62, 7000 Stuttgart 70, Federal Republic of Germany

Summary

A complex experiment was conducted to study the influence of two different basal media, BAP and the auxins 2,4-D and Dicamba in various concentrations on the induction and regeneration of calli derived from mature embryos of Lolium perenne. Data on the extent of regeneration and the number of regenerated plants are presented. Finally a protocol for induction and regeneration of embryogenic callus cultures in Lolium perenne is suggested.

Introduction

In a series of experiments the influence of various basal media, growth regulators and carbohydrate sources on the induction and regeneration of callus cultures in Lolium perenne as well as the influence of subculture intervalls were examined over the last three years. These parameters were varied over a wide range leading to a final experiment to confirm our previous results. The most promising combinations were tested and the data evaluated by statistical analysis.

Material and Methods

A split-plot experiment with two inbred lines as mainplots and a 3·3·2·2-factorial as subplots with partial confounding in blocks of 12 units was conducted in four replications.

The factorial treatments consisted of the genuine MS medium and a slight modification (thiamine concentration raised to 700 µg l^{-1}, no glycine, no pyridoxine and no nicotinic acid) as basal media, the auxins 2,4-D and Dicamba (7.5, 10.0 and 12.5 mg l^{-1}) and the cytokinine BAP (0.00, 0.25 and 0.50 mg l^{-1}) in all possible combinations. The media were solidified with agar (8 g l^{-1}) and adjusted to pH 5.8 prior to autoclaving. Filtersterilized maltose (30 g l^{-1}) was used as carbohydrate source throughout.

Ten chopped mature embryos were placed in each petri dish (55 mm) and incubated at 25 °C in dim light (approx. 300 lx) for four weeks then microscopically inspected and scored on a scale from 0 (no reaction at all) to five (excellent callus induction and growth). Only calli that scored 2 and better were transferred to fresh medium with halfed concentrations of the growth regulators and incubated for another four weeks under the same conditions.

Only calli with scores of 2 and above were subcultured for regeneration. The same basal medium as for the induction was used but supplemented with 0.1 mg l^{-1} of 2,4-D and BAP. The cultures were incubated for six weeks at 22 °C in bright fluorescent light (approx. 5 000 lx; 16 hrs photoperiod).

For the statistical analysis the proportion of regenerating calli was transformed according to the formula $z_i = \sqrt{\frac{y_i + 0.375}{n_i + 0.750}}$ (with z_i = transformed variable, y_i = number of regenerating calli, n_i = total number of calli per petri dish) and an weighted analysis of variance ($4[n_i + 0.5]$, the reciprocal of the expected value of the variance of z_i used as weight) was performed to obtain best linear unbiased predictors (BLUP). The square root of the number of regenerants was evaluated by analysis of covariance with the number of regenerating calli as concomitant variable. All results are given on the untransformed scale.

Results and Discussion

In the proportion of calli producing regenerants there was a marked difference between the two genotypes tested (13.1% against 2.8% on average). This proportion increased with the concentration of BAP but decreased with the concentration of auxin (see table 1). The effect of BAP was much more pronounced in one of the genotypes (from 2.7% to 20.7% vs. 1.9% to 3.2%; details *not shown*). In addtion there was a strong auxin-genotype interaction indicating that Dicamba is superior to 2,4-D with some genotypes (details not shown).

Table 1. Proportion of calli producing regenerants (%)

Auxin mg l^{-1}	BAP mg l^{-1} 0.00	0.25	0.50	Mean
7.5	3.8_b	11.3_{ab}	15.7_a	10.3_a
10.0	1.4_b	9.7_{ab}	10.7_a	7.3_a
12.5	1.6_b	7.6_{ab}	9.5_a	6.2_a
Mean	2.3_b	9.6_{ab}	12.0_a	7.9

Table 2. Number of regenerants produced per petri dish

Auxin mg l^{-1}	BAP mg l^{-1} 0.00	0.25	0.50	Mean
7.5	2.8_a	3.4_a	7.3_a	4.5_a
10.0	3.9_a	3.4_a	4.3_a	3.8_a
12.5	3.3_a	1.9_a	3.6_a	2.9_a
Mean	3.3_b	2.9_{ab}	5.0_a	3.7

Marginal means and means in lines followed by a common letter are not significantly different at the 5% level.

No difference in the number of regenerants (adjusted to the number of regenerating calli) could be observed between the two genotypes. The only factor that had a main effect on this character was the concentration of BAP (see table 2).

In general, the number of regenerants increased with the concentration of BAP. This effect could not be observed if either the genuine MS medium in combination with 2,4-D or the modified MS medium in combination with Dicamba was used. The increase in the number of regenerants on the modified MS medium supplemented with 2,4-D was only moderate (4.0 to 5.0) but was remarkable on the *genuine MS medium* combined with Dicamba (3.3 to 8.9; details not shown).

Our results suggest that for the induction of embryogenic calli from mature embryos in Lolium perenne the use of the genuine MS medium supplemented with 7.5 mg l^{-1} Dicamba in combination with 0.50 mg l^{-1} BAP and maltose instead of sucrose as the carbohydrate source is most suitable. The concentrations of the growth regulators should be reduced by 50% after four weeks and the regeneration should commence after a total induction period of eight weeks.

LONG TERM STORAGE AND IN VITRO MULTIPLICATION IN PERENNIAL RYEGRASS

Schmidt, F.[1] and Posselt, U. K.[2]

[1] Forschungsschwerpunkt Biotechnologie und Pflanzenzüchtung
[2] Landessaatzuchtanstalt, Universität Hohenheim
Postfach 70 05 62, 7000 Stuttgart 70, Federal Republic of Germany

Summary

Lateral buds of six Lolium perenne inbred lines 5 to 15 mm long were cultured on MS – medium supplemented with 0.2 mg l^{-1} kinetin. After four to six weeks the plantlets were well developed and could be stored in a cold room up to 18 month before subculture was neccessary.

For multiplication these plantlets were transferred to MS – media containing cytokinins in various concentrations and combinations. After five weeks the induced tillers were counted, separated, transferred to soil and grown in a greenhouse for six weeks. Tiller number, fresh and dry matter yield of the plants were determined and the dry matter content was calculated.
Keywords: Lolium perenne; tissue culture; BAP; Kinetin;
in vitro storage; in vitro multiplication

Introduction

During the developement of Gramineae numerous factors are involved in the control of tillering. Among these the distribution of assimilates as well as hormonal regulation seem to be of major importance.

Cytokinins exhibit a promoting effect on bud growth. Foliar or root application of 6-benzylamino-9-(tetrahydropyran-2-yl)-9H-purin (PBA) and 6-benzylaminopurine (BAP) to seedlings of barley, oat and wheat resulted in promoted growth of lateral buds (JOHNSTON & JEFFCOAT, 1977).

DALTON & DALE (1985) suggested the use of BAP for the multiplication of Lolium multiflorum plantlets derived from shoot tips in vitro.

A previous experiment carried out at our laboratory indicated that Lolium perenne may be propagated by organ culture as well.

Material and Methods

Tillers of young vigorously growing ryegrass plants were collected, surface sterilised in 70 % ethanol for ten seconds followed by immersion in 50% sodium hypochlorite solution for 15 minutes. Lateral buds 5 to 15 mm in size were excised aseptically and transferred to 30 ml culture tubes containing 10 ml of MS medium added with 0.2 mg l^{-1} of Kinetin.

The cultures were incubated at 22 °C in fluorescent light at an intensity of approximately 5 000 lx for 16 hours per day. After four weeks the regenerants had developed three to five leaves and were stored at 2 °C to 4 °C with an eight hour daily photoperiod at approximately 300 lx. After 12, 18 and 24 months the plantlets were returned to growing conditions for one week. Thereafter half of the plantlets were transferred to soil the others were subcultured on hormonfree MS medium and returned to storage conditions after four to six weeks.

For multiplication the plantlets were separated into individual tillers and transferred to MS media containing seven concentrations of

BAP and three concentrations of Kinetin in all combinations. The cultures were incubated at 22 °C unter intensive fluorescent light (12 000 lx). A completely randomised design in three (L 08), four (L 14) and five replicates (remaining genotypes) was used. After five weeks the plantlets were again separated into individual tillers and grown in a green house for six weeks. Tiller number, fresh and dry matter yield were determined and the dry matter content calculated.

Results and Discussion

During in vitro storage very few plantlets were lost by contamination. The survival after transfer to soil of plantlets stored 12 or 18 month was 85% to 92%, whereas some 50% of the plantlets stored for two years were lost suggesting regular subculturing every 12 (to 18) months.

In the in vitro multiplication experiment tillering could not be observed before the third week of culture. The analysis of variance showed a significant main effect of BAP concentration on all characters recorded, being strongly genotype dependend.
Kinetin had a direct influence on tiller bud elongation only when used alone, but reduced the effect of BAP in media containing both of them.
BAP applied as the only growth regulator proofed to be effective in promoting tillering (see table 1) without adversely affecting subsequent vegetative growth. The optimum concentration was in the range of 1.25 to 1.50 mg l^{-1}.

According to the pattern of tiller formation we assume that more tillers per plantlet (12 to 15) could be obtained. Besides a prolonged adaption from storage to growing conditions an extension of the culture period to eight weeks is recommended.

Table 1. Number of tillers produced by regenerants derived from lateral buds after five weeks of cultur on MS medium containing BAP.

BAP mg l^{-1}	Genotype L 02	L 07	L 08	L 14	L 29	L 30	Mean
0.00	1.0_b	1.4_b	1.1_a	1.0_b	1.0_a	1.4_b	1.2_b
0.50	4.2_a	6.0_a	3.7_a	3.0_{ab}	1.8_a	4.2_{ab}	3.9_a
1.00	1.2_b	4.2_{ab}	4.3_a	6.5_a	2.4_a	5.4_{ab}	3.9_a
1.25	2.2_b	4.8_{ab}	4.7_a	9.3_a	3.0_a	5.4_{ab}	4.7_a
1.50	2.2_b	7.8_a	3.7_a	4.5_{ab}	3.0_a	5.4_{ab}	4.5_a
1.75	2.2_b	4.6_{ab}	3.0_a	7.0_a	2.8_a	3.0_{ab}	3.7_a
2.00	2.6_b	3.8_{ab}	3.3_a	10.0_a	2.6_a	6.4_a	4.7_a
Mean	2.2_c	4.7_a	3.4_{ab}	5.9_a	2.4_{bc}	4.5_a	3.8

Means for genotypes and means in columns followed by a common letter are not significantly different at the 5% level

References

DALTON, S. J.; DALE, P. J. (1985). The application of in vitro tiller induction in Lolium multiflorum. Euphytica 34: 897–904

JOHNSTON, G. F. S.; JEFFCOAT, B. (1977). Effects of some growth regulators on tiller bud elongation in cereals. New Phytol. 79: 239–245

SELECTION OF LOLIUM MULTIFLORUM AND NITROGEN FIXING BACTERIA ASSOCIATED TO ITS RHIZOSPHERE

C. Scotti, G. Garau, M. Molinari

Istituto Sperimentale per le Colture Foraggere, 20075 Lodi, Italy

Summary

The aim of the present work is the research of coadapted N2 fixing bacteria (NFB) and grass plants (L. multiflorum) in order to constitute performing meadows with reduced N input. Fifty-two NFB strains, isolated in the rhizosphere of Italian ryegrass plants of permanent meadows, are studied for acetylene reduction activity (ARA) in pure culture and in association with young and adult plants of L. multiflorum. The results of the strains till now examined show a decrease in ARA both for values and for variability between strains from the pure culture to the association with adult plants.
Keywords: N2 fixing bacteria, rhizosphere, Lolium multiflorum, acetylene reduction activity.

Introduction

Our aim is the selection of coadapted bacteria and plants for the constitution of meadows, in pure stand or in mixture with legumes, highly performing with reduced mineral nitrogen input. Isolation of bacteria and selection of plants were made on the same parental populations: permanent meadow L. multiflorum plants with their associated N2 fixing microflora. Plant material coming from the same collection sites is resulted highly persistent and summer yielding and is now object of a specific breeding program at Lodi Institute (Rotili et al., 1987). As for bacteria we wanted to know the potential and the variability for ARA in pure culture and in association with plants.

Material and methods

NFB strains are isolated in the rhizosphere of L. multiflorum plants collected in permanent meadows 80-100 years old of the Lodi-Crema region in the Po Valley (Northern Italy). Isolation is carried out with the spermosphere model technique (Thomas Bauzon et al., 1982) using L. multiflorum cv. Crema, an experimental variety issued from the same population as the collected plants. ARA of isolated strains is studied in: a) pure culture on N-free medium supplied with C sources; b) association with young plantlets (14-22 days old), in tubes, on N-free medium supplied or not with C sources; c) association with adult plant in microplots 5 cm Ø x 50 cm high, filled with sterile sand (10 plants/plot); 2 levels of N (2.5 and 5 mM nitrate), in absence or presence of C sources. The results of four strains are presented: 2 probable Enterobacteriaceae, 1 probable Azospirillum, 1 not determined, and A. brasilense SpCd as tester.

Results

Fifty-two N2 fixing Gram-, catalase+, non sporing rods are isolated and classified into 19 groups. a) ARA in pure culture raises from 5 to 500 nmol C2H4/h; 12% of the strains, including SpCd, shows values between 350 and 500. b) ARA in association with plantlets is detectable (2-44 nmol C2H4/h/tube) only when C sources (C+) are present, while bacterial density is about the same in C- and C+. Addition of C sources in the C- tubes allows ARA detection (Martin & Glatzle, 1981). c) ARA in association with adult plants (data of the 1st cut), is measured on the intact plot and on excised roots 40 days after inoculation, at the end of the 1st productive cycle. ARA of intact plots is detectable only for 3 strains (129, 141, SpCd) with low values (0.77 and 5 total micromol C2H4 after 20 hours of incubation under 20% acetylene). Excised roots of all the 5 strains are found to reduce acetylene in C- treatments. No significant effect of inoculation is found neither in aerial, crown, roots D.M., nor in number of leaves. Nevertheless, in general, root D.M. is higher and shoot/root ratio lower in inoculated plots than in testers.

Discussion

The introduction of plant as "environment" for bacteria, even in controlled conditions for other environmental factors, influences bacterial population density and, to a greater extent, the process of N2 fixation. Our choice was to establish favourable conditions for the plant partner as for growth as for ARA measure (aerial part in open air). For a same strain, the measure of ARA in pure culture, in association with plantlets "in vitro", and finally in association with adult plants corresponds to a decrease of AR activity detected and to an increase of variability of results. Inter strains variability decreases starting from pure culture to the association with adult plants. Strains tested with adult plants seem to affect positively root D.M. rather than aerial D.M. This effect is found for all the strains studied.

Literature cited

Rotili P., Zannone L., Scotti C., Gnocchi G., Proietti S., (1987), "Agronomic and feeding value of L. multiflorum populations collected in the Po Valley", in INRA (ed): "Natural variation and breeding for adaptation", Lusignan, 79-86.

Thomas Bauzon D., Weinhard P., Villecourt P., Balandreau J., (1982), "The spermosphere model. I. Its use in growing, counting, and isolating N2 fixing bacteria from the rhizosphere of rice" Can. J. Microbiol., 28, 922-928.

Martin P., Glatzle A., (1981), "Mutual influences of Azospirillum spp. and grass seedlings", in W. Klingmüller (ed): "Azospirillum: genetics, physiology, ecology", Birkhauser Verlag, Basel, 108-120.

REGENERATION OF PLANTS FROM CALLUS AND PROTOPLASTS OF POA PRATENSIS L. (KENTUCKY BLUEGRASS)

P. van der Valk[1], M.A.C.M. Zaal and H.F. van Ark[2]

Centre for Plant Breeding Research (CPO), P.O. Box 16, 6700 AA Wageningen, the Netherlands

Introduction

Poa pratensis is a highly apomictic temperate grass species, used for both forage and turf (Åkerberg, 1939). The apomictic mode of reproduction hampers cultivar improvement by conventional breeding procedures. Genetic improvement by biotechnological techniques may become an appropriate alternative for apomictic grasses (Metzinger et al., 1987). The efficient regeneration of plants from cultured cells and tissues is a prerequisite for the successful application of biotechnology. The type of explant used to initiate *in vitro* cultures is an important factor determining the capacity of cells and tissues for plant regeneration. The highest frequencies of plant regeneration have been obtained from immature plant parts such as immature embryos and young inflorescences (Vasil, 1987). In this communication we summarize studies on i) the capacity of mature seeds and immature inflorescences of 16 cultivars to form embryogenic callus cultures and plants (Van der Valk et al., 1989); ii) the effect of abscisic acid (ABA) and gelrite on the tissue culture response of seed-derived callus cultures (Van Ark et al., 1990); iii) the initiation of suspension cultures and the culture of suspension-derived protoplasts (Van der Valk et al., 1988).

Materials and Methods

Only a brief description of Materials and Methods is given. For more details, see references 4-6.

Seeds and inflorescence segments were cultured on MS-medium supplemented with 0.4 mg/l thiamine-HCl, 30 g/l sucrose, 2 mg/l 2,4-D and 0.8% agar or 0.3% gelrite. Plant regeneration was induced on MS-medium devoid of 2,4-D. Suspension cultures were initiated from friable callus with shoot-forming ability or directly from mature embryos. Protoplasts were isolated from 4-5 days old suspension cultures and cultured in KM8P-medium. Protoplast-derived calli were cultured on MS-medium with or without additional sugars.

1. Present address: Van der Have Research, P.O. Box 1, 4410 AA Rilland, the Netherlands
2. Present address: Barenbrug Holland B.V., Stationstraat 40, 6678 AC Oosterhout, the Netherlands

Results

Callus induction and plant regeneration

Both inflorescences and seeds formed 3 types of callus on 2,4-D supplemented MS-medium: i) watery and smooth; ii) compact; iii) friable. Plants could be regenerated from both compact and friable callus. Plant regeneration took place mainly through organogenesis. Somatic embryos were frequently present on the surface of compact calli. Germination of somatic embryos was occasionally observed. The morphogenic response of immature inflorescences was much higher than that of seeds. Plants were recovered from 14 cultivars at a high frequency (up to 79% of the callus cultures) when inflorescences were used and from only 3 cultivars at a low frequency (up to 3%) with seeds. Plants were generally green, but albino shoots developed occasionally from friable callus. Attempts were made for practical reasons to enhance the tissue culture response of seed-derived calli. The use of gelrite instead of agar significantly increased the frequency of shoot regeneration. Addition to regeneration media of ABA, known to increase shoot formation from callus in a number of species (see reference 4), stimulated the formation of green spots and somatic embryos, but failed to increase shoot regeneration.

Protoplast isolation and culture

Albino plantlets could be regenerated from suspension cells of 2 cultivars. Protoplasts, isolated from these suspension cultures, were able to form microcalli. These calli formed somatic embryos and albino shoots upon transfer to sorbitol-supplemented MS-medium.

Conclusion

High frequency plant regeneration was obtained from young inflorescence-derived callus cultures of several Poa pratensis cultivars. These plants may contain useful genetic (somaclonal) variation (Taliaferro et al., 1989). Further studies are needed in Poa pratensis to optimize a protoplast-to-plant system amenable for genetic tranformation studies.

References

1 Åkerberg, E. (1939). Hereditas 25:359-370.
2 Metzinger, B.D., C.M. Taliaferro, B.B. Johnson & E.D. Mitchell (1987). Plant Cell, Tissue and Organ Culture 10:31-38.
3 Taliaferro, C.M, S.M. Dabo, E.D. Mitchell, B.B. Johnson & B.D. Metzinger (1989). Plant Cell, Tissue and Organ Culture 19:257-266.
4 Van Ark, H.F., M.A.C.M. Zaal, J. Creemers-Molenaar & P. van der Valk (1990). In preparation.
5 Van der Valk, P., M.A.C.M. Zaal & J. Creemers-Molenaar (1988). Euphytica S:169-176.
6 Van der Valk, P., M.A.C.M. Zaal & J. Creemers-Molenaar (1989). Plant Cell Reports 7:644-647.
7 Vasil, I.K. (1987). J. Plant Physiol. 128:193-218.

CYTOLOGICAL MECHANISMS RESPONSIBLE FOR 2N POLLEN PRODUCTION IN PERENNIAL RYEGRASS (LOLIUM PERENNE)

M. Wagenvoort, M. de Vries-van Hulten, G.D. Winkelhorst and A.P.M. den Nijs

Centre for Plant Breeding Research CPO, POB 16, 6700 AA Wageningen, the Netherlands

Summary

Pollen grain diameter was measured to estimate the proportion of 2n pollen of five genotypes of *Lolium perenne*, selected before as 2n pollen producers. Most genotypes had different pollen grain sizes.
Meiotic studies revealed two restitution mechanisms in PMCs. Firstly, in two genotypes non-disjunction at second Metaphase of meiosis occurred followed by the suppression of the formation of a new cell wall.
Secondly, in one genotype premeiotic doubling was observed. Both meiotic aberrations would result in the formation of diploid and probably also polyploid pollen grains. Seed set in 4x x 2x crosses was evaluated as a check of 2n pollen production.
Keywords: meiotic mutants, 2n pollen, premeiotic doubling, *Lolium*.

Introduction

Tetraploid cultivars of perennial ryegrass are gaining popularity as fodder and pasture crop in north-western Europe. Up to now new tetraploids are created by colchicine-doubling. This method of polyploidization has several disadvantages of which inbreeding, low fertility, and the occurrence of mixoploids are most important. Sexual polyploidization is used to generate new tetraploids (Den Nijs and Stephenson, 1988). This method relies on numerically unreduced (2n) gametes resulting from aberrant meiotic divisions (Sala et al., 1989). This contribution reports the preliminary results of meiotic studies and additional interploidy crosses.

Material and methods

Pollen grains of five field grown genotypes of perennial ryegrass, selected before in diverse diploid inbred populations, were stained in a mixture of 100 ml lactophenol and 8 ml 1% acid fuchsin in water. The diameters of at least 200 well-stained pollen grains per genotype were measured. The percentage of 2n pollen was estimated from frequency distributions of pollen grain size. Pollen grains with a diameter above 38.5 μm were assumed to be 2n.
Spikes were fixed in Carnoy's solution for 24 hrs, and Pollen Mother Cells (PMCs) stained with aceto-orcein, for meiotic studies. Seeds from 4x x 2x crosses were harvested on the tetraploid only.

2n Pollen and crosses

Table 1 presents the origin of five genotypes and the results of 2n pollen measurements and 4x x 2x crosses.

Table 1. Origin, pollen stainability (PS), percentage of 2n pollen and seed set in 4x x 2x crosses of 5 diploid genotypes of ryegrass.

Genotype	Origin	PS (%)	2n pollen	Seed set[a]
1180	Netherlands	93.0	0.0	16.4 (5)
2190	Wales	62.5	56.0	-
3069	Czechoslovakia	62.7	38.0	2.7 (3)
5210	New Zealand	49.6	2.0	12.8 (4)
6529	New Zealand	83.7	21.2	15.7 (6)

[a] mean number of seeds per spike; ( ) the number of combinations.

There was no relation between the frequency of 2n pollen and seed set in the interploidy crosses. This was probably due to the occurrence of some selfing in the tetraploids or to the variability of the 2n pollen frequency in the diploids (Jansen and den Nijs, this symposium).

Meiotic studies

Table 2. Meiotic aberrations of 5 diploid genotypes of ryegrass.

Genotype	Metaphase I			Metaphase II	Sporad stage
	N	Bivalents	Univalents		
1180	13	6.8	0.5	non-disjunction + aberrant cytokinesis	dyads + tetrads
2190	10	3.1	8.7	"	dyads + triads + tetrads
3069	9	6.8	0.4	-	-
5210	29	6.7	0.5	-	tetrads only
6529	-	diploid, tetraploid and possibly octoploid cells		- 279 PMCs →	dyads 3%, triads 1% tetrads(n) 72% tetrads(2n) 24%

Non-disjunction at MII followed by aberrant cytokinesis is genetically equivalent to Second Division Restitution (SDR). In genotype 6529 both the normal diploid and the giant PMCs endured a normal meiosis, leading to distinctive genetic consequences compared to those of SDR. The restitution mechanisms found in some genotypes of ryegrass hold promise for the production of tetraploid populations with greater uniformity.

References

Den Nijs, A.P.M. & A.G. Stephenson, 1988. In: M. Cresti, P. Gori & E. Pacini (Eds.): Sexual reproduction in higher plants. Springer-Verlag, Berlin p. 131-136.

Sala, C.A., E.L. Camadro, M.T. Salaberry & A.O. Mendiburu, 1989. Euphytica 43: 1-6.

SOMACLONAL VARIATION IN LOLIUM MULTIFLORUM LAM. AND L. PERENNE L.

Z. Zwierzykowski, E. Zwierzykowska, W. Irzykowski

Polish Academy of Sciences, Institute of Plant Genetics, Poznan, Poland

Abstract

The objectives of this research were to: a) initiate callus cultures and regenerate plants from callus in L. multiflorum and L. perenne, b) evaluate somaclonal variation both in the SC1 regenerated plants of the two Lolium species and in the SC2 progenies of tetraploid regenerants originating from diploid L. multiflorum.

Callus cultures were induced from immature inflorescences (1-15 cm in length) of field-grown plants of L. muliflorum 2n=2x=14 (cv. Tur and Skrzeszowicki) and L. perenne 2n=2x=14 (cv. Arka and Gazon). Murashige and Skoog (1962) medium (MS) with different levels of 2,4-dichlorophenoxyacetic acid (2,4-D) was used for callus initiation (4.0 mg/L), maintaining (2.0 mg/L) and plant regeneration (0.2 mg/L).

There was considerable variation in the capacity of various genotypes to initiate callus and regenerate plants from callus. The callus induction frequency varied from 17.4 to 100 % in the studied genotypes of L. multiflorum cv. Tur.

Variation of different characters such as plant height, size and shape of leaves and spikes, tillering capacity, heading date, fertility and chromosome number was observed among the SC1 plants of the both species.

The chromosome number of all analyzed plants derived from L. perenne cv. Arka was 2n=14. The majority of SC1 plants from the both L. multiflorum cultivars and from L. perenne cv. Gazon had unchanged chromosome number (2n=2x=14). However, significant part of them (7.3 to 29.2 %) had the doubled chromosome number (2n=4x=28).

The considerable majority of diploid and tetraploid SC1 plants had dehiscent anthers with very high pollen viability. However, a great variation of seed set in the both groups of regenerated plants was observed. In L. multiflorum percentage of seed set was lower for tetraploids (2.3 to 43.8 %) compared to diploids (22.7 to 78.9 %).

Plants of the SC2 generation originating from 10 tetraploid regenerants of L. multiflorum cv. Tur showed wide variation in several morphological characters such as plant height, inflorescence length, number of generative tillers, 1000-seed weight, as well as in chromosome number and fertility. L. multiflorum (2n=28) cv. Kroto was used as the control. The variation of morphological characters within the SC2 population was greater than in the control. However, vigour of the SC2 plants was decreased.

In the SC2 plants pollen stainability was very high (on average 87.9%), seed set was significantly lower (on average 41.9 %) compared to the control (72.9 %) and varied from 1.0 % to 65.3 %. The 1000-seed weight varied significantly (from 2.97 to 5.97 g) and the mean value (4.44 g) was lower than in the control (5.53 g).

All analyzed plants in the SC2 progeny of diploid regenerants of L. multiflorum had 2n=14 chromosomes. In the SC2 generation originating from tetraploid regenerants of L. multiflorum the chromosome number varied from 2n=25 to 2n=32 with the domination of tetraploid plants (2n=28). Aneuploid plants frequency was high in some analyzed progenies.

BREEDERS SONG

ALL YOU NEED IS BREED (The breeder's lovesong)

(Free after The Beatles,
by van Wijk and van Wijk)

Feed, feed, feed
Seed, seed, seed
Breed, breed, breed

Breeding is the basis of all life
Without breeding man cannot survive
Towards better seed we all do strive, strive
Breeding is the basis of all life

CHORUS

All you need is breed
Ratatatata
All you need is breed
Ratatatata
All you need is breed seed
Breed is all you need

But for breeding you will have to meet
Without meeting thoughts cannot succeed
It is breeding friends that we all need, need
So towards this meeting we do speed

CHORUS

Be one dressed in suit or pair of jeans
In one's dotage, middle age or teens
It doesn't matter, we all transform genes, genes
Even if we don't know what it means

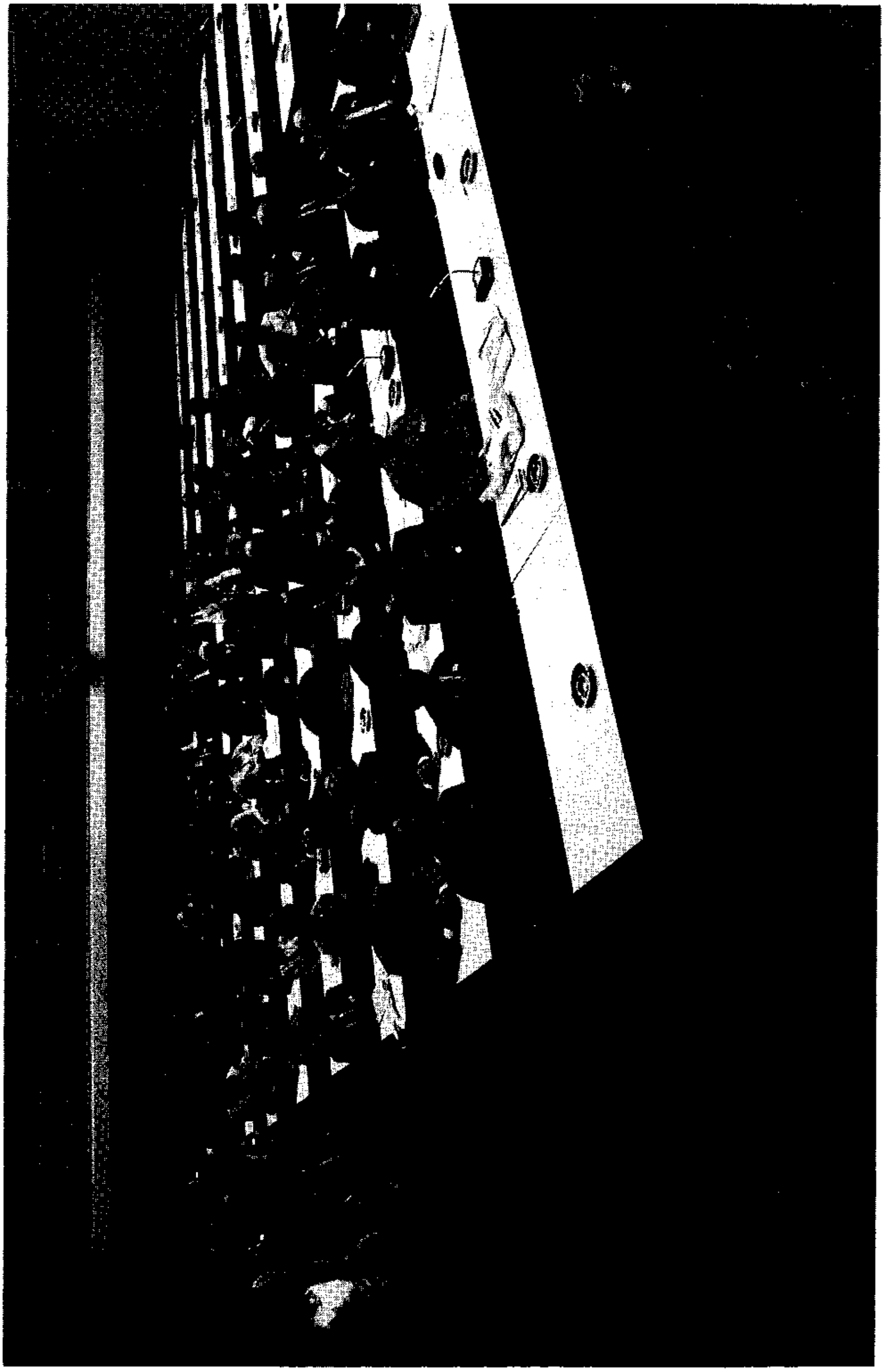
CHORUS

Biotechnics is the latest scoop
From baby bottoms to the newest soup
to biotechnics all scientists look, look
but WE don't want to end up in a tube

CHORUS

We look for the grasses highest yield
With feet firmly planted in the field
Fodder secrets are to us revealed, vealed
Seedbags are our mostly treasured shield.

CHORUS



LIST OF PARTICIPANTS

Austria
Betzwar, W.
(mechanization of field trials)
Wintersteiger Seedmech
Rechte Bahng. 30-32
A 2030 VIENNA

Belgium
Reheul, D.
(grass breeding)
Rijksstation voor
Plantenveredeling
Burg. van Gansberghelaan 109
9220 MERELBEKE

Canada
Faris, M.A.
MacGill University
CEMARP of McGill
University,
Centennial Centre, Room
141,21,111 Lakeshore Road
STE. ANNE DE BELLEVUE,
QUEBEC H9X
ICO

Cyprus
Hadjichristodoulou, A.
(breeding of field crops)
Min. of Agr./Nat. Resources
Agricultural Research Institute
NICOSIA

Denmark
Andersen, S.B.
(haploids in breeding)
Agr.Un./Dept. of Agr. Science
Thorvaldsensvej 40
1871 FREDERIKSBERG C

Kleinhout, A.
(grass breeding)
Danish Plant Breeding
Hojerupvej 31
DK-4660 ST HEDDINGE

Finland
Kajaste, S.
(forage breeding)
Hankkija Plant Breeding
Institute
SF-04300 HYRYLA

Miettinen, E.J.
(population genetics)
Agricultural Research Centre
Kainuu Research Station
SF-88600 SOTKAMO

France
Bayle, B.
(turf/forage breeding)
Green Genetics
B.P. 2
49320 LES ALLEUDS

Bourdon, P.
(forage breeding)
S-A Carneau Firus
B.P. 8
59310 ORCHIES

Carre, C.
(grass breeding)
Semunion Gazon
B.P. 9
77390 GUIGNES

Dattee, Y.
(Medicago environment DUS
methodology)
GEVES
La Miniere
78285 GUYANCOURT

Gensollen, V.
(variety testing)
GEVES
La Miniere
78280 GUYANCOURT

Ghesquiere, M.
(genetics/breeding methods)
Inst. Nat. Rech. Agro./St.
d'Amel.
des Plantes Fourr.
86600 LUSIGNAN

Grand-Ravel, C.
(ryegrass breeding)
INRA, Station d'Amelioration
des
PlantesDomaine de Crovelle
63039 CLERMONT
FERRAND

Hanja van, N.
(breeding forage grasses)
Barenbrug Holland bv
341, Avenue de Toulouse
82600 VERDUN SUR
GARONNE

Hansen, F.L.
(grass breeding)
Dansk Planteforaedling
Pousigny, Celle l'Evescault
86600 LUSIGNAN

Lallimand, J.
(electrophoresis)
GEVES
B.P. 52
17700 SURGERES

Lumaret, R.
(genetics, polyploidy, Dactylis)
Centre Emberger-CNRS
Route de Mende/B.P. 5051
34033 MONTPELLIER
CEDEX

Mousset-Declas, C.
(genetics/plant breeding)
INRA-Genetique et
Amelioration des
Plantes, B.V. 1540
21034 DIJON CEDEX

Perchet, M.
RAGT 18, Rue de Seguret
Saincric
12000 RODEZ

Poisson, C.
(interspecific hybrids,
Festulolium)
Institut Nat. de la Rech.
Agro./Stat. d'Amelioration des
Plantes Fourr.
86600 LUSIGNAN

Woude van der, K.
(grass breeding)
Van der Have France
Domaine de la Revanche
32700 LECTOURE

Germany
Berner, P.
(grass breeding)
Saatzucht Steinach GmbH
Wittelsbacher Strasse 15
D-8441 STEINACH

Bertling, U.
Inst. for Agronomy and Plant
Breeding
Von Siebold Strasse 8
34 GOTTINGEN

Eickmeyer, F.
Institut fur Angewandte
Genetik
Herrenhauser Strasse 2
3000 HANNOVER 21

Entrup, E.L.
(grass breeding)
Deutsche Saatveredelung
Weissenburgstrasse 5
D-4780 LIPPSTADT

Feuerstein, U.
(grass breeding)
Deutsche Saatveredelung
Hof Steimke
D-2811 ASENDORF

Freudenstein, K.H.
(DUS variety testing)
Bundessortenamt
Osterfelddamm 80
3000 HANNOVER 61

Kobabe, G.
(breeding of hybrid varieties)
Inst. for Agronomy/Plant
Breeding Von Siebold Strasse 8
34 GOTTINGEN

Matzk, F.
(apomixis, interspecific hybrids)
Zentralinst. fur
Gen./Kulturpflanzenfo.
Corrensstrasse 3
GATERSLEBEN 4325

Netzband, K.
(grass breeding)
Institut fur Futterproduktion
Gutshof 7
1551 PAULINENAUE KREIS
NAUEN

Paul, C.
(breeding for quality)
Fed. Res. Centre Agr./Inst.
Grassland
Bundesallee 50
D-3300 BRAUNSCHWEIG

Posselt, U.K.
(forage crop breeding)
University of
Hohenheim/Landessaatzucht
O.O. Box 700562
D-7000 STUTTGART-70

Scheller, H.
(fodder crops breeding)
Bayerische Landesanst.
Bodenk/Pflanzb.
Vottinger Strasse 38
8050 FREISING

Schmidt, F.
(tissue culture forage grasses)
University of
Hohenheim/Landessaatzucht
P.O. Box 700562
D-7000 STUTTGART-70

Simon, U.
(fodder plant breeding)
Lehrstuhl fur Grunland u.
Futterbau
Technische Universitat
Munchen
D-8050
FREISING-WEIHENSTEPHA
N

Uhlmann, A.
(fodder crop breeding/seed
production
Deutsche Saatzuch AG
Clara-Zetkin Strasse 1
4300 QUEDLINBURG

Hungary
Bocsa, J.
(lucerne breeding)
GATE Agriculture Research
Institute
KOMPOLT H-3356

Iceland
Helgadottir, A.
The Agricultural Research
Institute
Keldnaholt
112 REYKJAVIK

Italy
Benvenuti, B.
(agricultural technology)
National Research Council
(CNR) Piazzale Aldo Moro
ROME

Caredda, S.
(forage crops/seed production)
Ist. Agronomia, Un. Sassari
Via E. de Nicola 07100
SASSARI

Falcinelli, M.
(breeding fodder crops)
Un. Studi di Perugia/Ist. di
Migl.
Gen., Veg., Facolta di Agraria
BORGO XX GIUGNO -
06100 PERUGIA

Floris, R.
(germsplasm eval. by molecular
markers)
Centro Miglioramento Prod.
Pascoli
CNR
Via Enrico de Nicola
07100 SASSARI

Mariani, A.
(cytogenetics)
Centro di Studio
Miglioramento
Borgo XX Giugno 74
06100 PERUGIA

Piano, E.
(forage breeding)
Ist. Sperim. per le Colture
Foraggere
Viale Piacenza 29
20075 LODI

Romani, M.
(grass breeding/seed
multiplication)
Ist. Sperim. per le Colture
Foraggere
Viale Piacenza, 29
20075 LODI

Rotili, P.
Istituto Sperimentale per le
Colture Foraggere
Viale Piacenza 29
20075 LODI (MI)

Scotti, C.
(breeding lucerne/forage
grasses)
Ist. Sperim. per le Colture
Foraggere
Viale Piacenza, 29
20075 LODI

Tavoletti, S.
(cytogenetics)
Centro di Studio per il
Miglioramento
Genetico delle Plante
Foraggere
BORGO XX GIUGNO 74 -
PERUGIA

Veronesi, F.
(plant breeding)
University of Sassari
Via E. de Nicola
07100 SASSARI

Japan
Fujimoto, F.
(plant breeding in rel. to
adaptation)
National Grassland Research
Institute
768 Senbonmatsu
NISHINASUNO, TOCHIGI
329-27

Netherlands
Ark van, H.F.
(biotechnology)
Barenbrug Holland bv
Duitsekampweg 60
6874 BX WOLFHEZE

Beerepoot, L.J.
(grass breeding)
Barenbrug Holland bv
Duitskampweg 60
6874 BX WOLFHEZE

Bogaert van den, C.
(grass breeding)
Zelder bv
P.o. Box 26
6590 AA GENNEP

Creemers-Molenaar, T.
(plant cell biology)
Barenbrug bv
Duitskampweg 60
6874 BX WOLFHEZE

Cruchten van, C.
(grass breeding)
Green Genetics bv
P.O. Box 2
9679 ZG SCHEEMDA

Degenaars, G.
(perennial ryegrass)
University of Amsterdam
Kruislaan 38
1098 SM AMSTERDAM

Dijk van, G.E.
(grass breeding)
Pr. Bernhardlaan 5
6721 DN BENNEKOM

Dijkstra, J.A.
(grass breeding)
Barenbrug Breeding Station
Duitskampweg 60
6874 BX WOLFHEZE

Elgersma, A.
(grass seed production)
CPO
P.O. Box 16
6700 AA WAGENINGEN

Ghijsen, H.C.
(breeding)
Barenbrug Research
Duitskampweg 60
6874 BX WOLFHEZE

Hintzen, J.
(grass breeding)
Mommersteeg International bv
P.O. Box 1
5250 AA VLIJMEN

Joordens, J.
(fodder
grammeae,legumes,crucifera)
J. Joordens Zaadhandel bv
P.O. Box 7823
5995 ZG KESSEL

Joordens, M.
(fodder
crucifera,grammineae,legumes)
J. Joordens Zaadhandel bv
Postbus 7823
5995 ZG KESSEL

Kamps, M.
(breeding/registration variety)
Dutch Breeders Association
NZP
P.O. BOX 516
3800 AM AMERSFOORT

Klein Geltink, D.J.A.
(DUS testing)
CRZ
P.O. Box 32
6700 AA WAGENINGEN

Klooster van 't, G.J.M.
(breeding)
Barenbrug Holland bv
Duitskampweg 60
6874 BX WOLFHEZE

Lantinga, E.
(grassland husbandry)
Department of Field
Crops/Grassland Sc.
Haarweg 333
6709 RZ WAGENINGEN

Maas van der, H.
(plantcel biology)
CPO
Postbus 16
6700 AA WAGENINGEN

Mannetje 't, L.
(grasses, legumes)
Dep.
Landbouwpl./Graslandkunde
LU, Haarweg 333
6709 RZ WAGENINGEN

Meer, van der H.G.
(forage production/nitrogen
cycle)
Centre for Agrobiological
Research
P.O. Box 14
6700 AA WAGENINGEN

Mulder, G.H.
(production/maintenance of
varieties)
NAK, Postbus 51
6717 BB EDE

Neele, A.E.F.
(grass breeding)
Van Engelen Zaden bv
Postbus 35
5250 AA VLIJMEN

Neuteboom, J.
Vakgroep
Landbouwplantenteelt en
Graslandkunde LU
Haarweg 333
6709 RZ WAGENINGEN

Nienhuis, K.H.
(DUS variety testing)
CRZ
P.O. Box 32
6700 AA WAGENINGEN

Nijs den, A.P.M.
(grass breeding)
Centre for Plant Breeding
Research
P.O. Box 16
6700 AA WAGENINGEN

Reijden, van der, A.
(grass breeding)
Green Genetics bv
Stationsstraat 124
9679 EG SCHEEMDA

Reiling, R.
(plant breeding)
CEBECO Zaden bv
Postbus 139
8200 AC LELYSTAD

Valk van der, P.
(biotechnology)
Van der Have Seeds
P.O. Box 1
4410 AA RILLAND

Vijn, R.
(grass breeding)
Van Engelen Zaden bv
P.o. Box 35
5250 AA VLIJMEN

Visscher, J.
(variety testing)
CRZ
P.O. Box 32
6700 AA WAGENINGEN

Visser de, R.
(energetics/environmental-physi
ology)
CABO
P.O. Box 14
6700 AA WAGENINGEN

Vuuren van, A.
(ruminant nutrition)
Research Inst. for
Livest./Nutrition
P.O. Box 160
8200 AD LELYSTAD

Wagenvoort, M.
(cytogenetics)
Centre for Plant Breeding
Research
P.O. Box 16
6700 AA WAGENINGEN

Westerdijk-Hoks, I.B.P.
(grass breeding)
Cebeco Zaden bv
P.O. Box 139
8219 PR LELYSTAD

Wijk van, A.J.P.
(grass breeding)
Van der Have Research/Grass
Breed. Dep.
Postbus 127
5250 AC VLIJMEN

Norway

Aasmo, M.
(legume breeding)
Norwegian State Agr. Res.
Station
P.O. Box 100
N-9001 TROMSO

Larsen, A.
(breeding of winter hardiness
in grass)
Norwegian State Agr. Res.
Station
Vagones Research Station
N-8000 BODQ

Marum, P.
(legume breeding/variety
testing)
Loken Researcvh Station
2940 HEGGENES

Myhre, A.
(population genetics)
Agricultural University of
Norway
Box 40
N-1432 AS-NLH

Opsahl-Ferstad, H.G.
(embryogenesis/anther culture)
Agricultural University of
Norway
Box 40
N-1432 AAS-NLH

Rognli, O.A.
(genetics/breeding research)
Agricultural University of
Norway
P.O. Box 40
N-1432 AAS-NLH

Schjelderup, I.
(forage grass breeding)
Agr. Research Council of
Norway
Box 100
N-9001 TROMSO

Simonsen, O.
(forage breeding/biotechnology)
Agr. Res. Council/Biotechn.
Progr.
P.O. Box 51
N-1432 Aas-NLH

Poland

Mlyniec, W.
(forage plant breeding)
Polish Academy of Sciences
ul. Nowy Swiat 72
00-330 WARSAW

Pronczuk, S.
(plant breeding)
Plant breeding/Accl. Institute
Radzikow
BLONIE 05-870

Solgala M.
(plant breeding)
Instytut Hodowli I
Aklimatyzacji
Roslin
RADZIKOW, 05-870 BLONIA
(WARZAW)

Staszewski, Z.
(perennial fodder crops
breeding)
Plant Breeding/Acclimatization
Inst.
Radzikow
BLONIE 05-870

Zwierzykowski, Z.
(cytogenetics/tissue culture of
grasses)
Polish Academy of Sciences
Strzeszynska 34
60-479 POZNAN

Spain
Oliveira Prendes, J.A.
(grass breeding)
Centro de Investigaciones
Agrarias de
Mabegondo, Apdo 10
15080 LA CORUNA

Sweden
Andersson, B.
(breeding of red clover)
Svalof AB
S-26800 SVALOV

Lindvall, E.
(grass breeding)
Svalof AB, Norrlandsavd
Box 5097
S-90005 UMEA

Nilsson, I.
breeding red clover)
Svalof AB
Dag Hammarskjolds vag 180
S-75651 UPPSALA

Sjodin, J.
(breeding forage crops)
Svalof AB
26800 SVALOF

Switzerland
Badoux, S.
(plant breeding)
Federal Research Station for
Agricult.
Changins
CH-1260 NYON

Boller, B.C.
(clover/ryegrass breeding)
Eidgen. Forsch. Anstalt
Pflanzenbau
Reckenholz, Postfach
CH-8046 ZURICH

United kingdom
Ball, T.
(ryegrass/fodder brassicas)
Twyford Seeds Ltd
Kings Sutton
BANBURY, OXFORDSHIRE
OX173QW

Camlin, M.S.
(variety evaluation)
Dep. of Agr.,Plant Testing
Station
Crossnagreevy
BT6 9SH BELFAST

Evans, G.M.
(cytogenetics/plant breeding)
University College of Wales
Department of Agricultural
Sciences
PENGLAIS, ABERYSTWYTH
SY 233DD

Fox, K.S.
(grass breeding)
Booker Seeds Limited
Boston Rd
NG34 7HA SLEAFORD
LINCS

Hayward, M.D.
(plant breeding genetics)
Welsh Plant Breeding Station
Plas Gogerddan
ABERYSTWYTH, DYFED
SY23 3EB

Humphreys, M.O.
(grass breeding)
AFRC, IGAP, Welsh Plant
Breeding
Station
ABERYSTWYTH, DYFED
SY23 3EB

Rhodes, I.
(plant breeding hecophysiology)
IGER, Welsh Plant Breeding
Station
Plas Gogerddan
ABERYSTWYTH DYFED
SY23 3EB

Strickland, T.
(grass varieties/productions
Danish Plant Breeding Ltd
Didbrook Fields
TODDINGTON
CHELTENHAM GLOS GL54
5PE

Wilkins, P.W.
(grass breeding)
IGER, Welsh Plant Breeding
Station
Plas Gogerddan
ABERYSTWYTH, DYFED
SY23 3EB

Wilson, D.
(stress tolerance,herbage
quality)
AFRC, IGER, Welsh Plant
Breeding
Station ABERYSTWYTH,
DYFED SY23 3EB

United states

Conger, B.
(biotechnology)
University of Tennessee
P.O. Box 1071
KNOXVILLE TN 37901-1071

Yugoslavia

Katic, S.
(lucerne breeding)
Institute of field and Vegetable
Crops
Maksima Gorkog 30
21000 NOVI SAD

AUTHOR INDEX

Senior authors are printed bold.

SUBJECT INDEX