Use of unreduced gametes of diploid potato (*Solanum tuberosum* L.) for true potato seed production through 4x-2x crosses

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Subject headings: Unreduced gametes, *Solanum tuberosum*, true potato seed breeding, 4x-2x crosses, bacterial wilt resistance, parental selection.

Bibliographic Abstract: The thesis describes the genetic bases of 2n-pollen formation. A comparison was made of the frequencies of 2n-pollen production in a different environment using the progeny of diallel crosses between five well-known diploid clones and three clones of *Solanum phureja*. It was evident that this character has a complex type of inheritance; through repeated cycles of selection for 2n-pollen a four-fold increase could be achieved at population level. Through proper selection of diploid male parents it was established that 2n-pollen genotypes can be effective for transferring useful characters, like bacterial wilt resistance, in 4x-2x crosses. The progenies derived from 4x-2x, 4x-4x and 4x-selfings were compared to find the optimal breeding approach for the production of TPS varieties. There were clear indications that it was possible to breed TPS varieties directly by 4x-2x crosses. This means that the advantages of breeding at diploid level and the effective transfer of desirable traits by 2n-gametes can be used in the TPS breeding.

Propositions (Stellingen)

1. Despite the complicated genetic control and great influence of environment on 2n-pollen formation in potato, it is still possible to use 2n-pollen for practical breeding (this thesis; Veilleux & Lauer, 1981. Theor. Appl. Genet., 59:95-100).

2. The frequencies of 2n-pollen can be increased through recurrent selection, but it requires great efforts (this thesis; Ortiz & Peloquin, 1992. J. Genet. & Breed. 46:383-390).

3. Although genetic engineering is potentially promising for genetic improvement of existing potato varieties, the use of 2n-gametes is promising for the efficient production of new varieties in which diploid wild species are involved (this thesis; Watanabe, 1994. in J.E. Bradshaw & G.R. Mackay (Ed). Potato Genetics, Mol. genet. pp 213-229).

4. In breeding for TPS varieties, it is important to screen many agricultural traits in seedling as well as clonally propagated population (this thesis).

5. Sexual polyploidization simulates procedures of natural evolution of auto- and allo-polyploid crops (this thesis; Bingham, 1980. in W.H. Lewis (Ed). Polyploidy, Biological Relevance pp 471-489).

6. Contribution to social progress does not depend on how much you have promised but how much you have done.

7. History teaches you to be experienced, Mathematics tells you to be smart and potato genetics reminds you that life is short.

8. Sustainable agriculture can not be established unless sufficient number of students retain in agricultural sciences after graduation.

9. There is a popular song in Chinese that says, it is not because I could not understand what is happening in the world, but it is because the world is changing so quickly.

Stellingen behorende bij proefschrift getiteld **"Use of unreduced gametes of diploid potato (*Solanum tuberosum* L.) for true potato seed production through 4x-2x crosses"** by Qu Dongyu

Wageningen, Febuary 12, 1996.

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In completing this thesis, I have fulfilled the requirement of the Wageningen Agricultural University. This could not have been achieved without the support of numerous persons, from Wageningen and China, on whom I depended.

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CONTENTS

Chapter 1

General introduction

1.1 History of potato

Potato has been in cultivation in South America for almost ten thousand years (Hawkes, 1992), and became a world crop relatively recently. The plant first entered Europe during the last quarter of the sixteenth century and the approximate date is supposed to be 1571. But there were probably two introductions into Europe, one into Spain, in 1570 and the other into England in 1590. When potato entered Europe, it was adapted to the short day conditions (12-hour day) of the Andes and did not tuberize in the long day conditions (16-18-hour day) of the temperate Europe. The first introductions were the Andean tetraploid potato ($2n=4x=48$, *Solanum tuberosum L, subspp. andigena* Hawkes). As a result of unconscious selection during a few centuries, it became adapted to the long day conditions and spread out as a tuber crop into central and eastern Europe. This crop is the present day potato, *S. tuberosum L., subspp. tuberosum*. Among the most important world crops, potato occupies the fourth place in terms of growing area, distribution and production when compared to wheat, rice and maize.

1.2 Potato in China

It was the Dutch who introduced potato into China in 1619-1662 when they occupied Taiwan during this period. Songxi county of Fujian province was the first place on mainland to grow potato before 1700 (Songxi county Archives, 1700). Later on potato was introduced from Korea, Japan and Russia into Northeastern China, from Germany into Shandong, from France and Belgium into Shanxi, Shaanxi and Gansu, from England, America and Canada into Sichuan (Yang Hongzu, 1983).

Before 1950s the potato growing area in China was about 300,000 ha. Nowadays it has reached three million ha. The top twelve potato growing provinces are listed below: They are predominantly located in the single cropping areas, either in humid or arid regions. Diversity of environments made potato to be grown all year round in China. The major cultivation regions are classified into:

Single Cropping area including Northeast China, Northwest China and highland (1500m ASL) in Southwest China which accounted for 85% of the total area.

Double cropping area including Northern China plain, Yangtze River area and lowlands of Southwest China which amounts to 12%.

Winter cropping area including Southern China provinces like Fujian, Taiwan, Guandong, Guanxi and Hainan, occupying about 3% of the total growing area.

Province	Potato growing area in 1000 ha (approximately)
Sichuan	340
Gansu	300
Helongjiang	200
I.Mongolia	200
Shanxi	200
Shaanxi	200
Yunnan	180
Hubei	170
Hebei	100
Xinjiang	100
Qinghai	80
Liaoning	80

1.3 Potato in developing countries

In the developing world potato production is mainly confined to the subtropical lowlands that are comparable to temperate highlands and double cropping regions of China (CIP report, Table 1.1). Although potato has been introduced into lowland tropical countries more than a century ago, locally selected potato cultivars were few until recently. Substantial production in these areas depended, and still depends, on the cultivars of the northern temperate regions of Europe or Northern America. Some of the old varieties of these regions, which are still in cultivation, are given in Table 1.2. They range from the Russet Burbank released in the USA in 1876 upto Epoka selected in Poland in 1955. Not unexpectedly, these cultivars produce low yields and are unable to meet the requirements of present day competitive agriculture.

Besides directly cultivating old European and North American cultivars, they have also been used extensively as parents in breeding local cultivars. For example, a number of cultivars from Eastern Europe (Poland, former East Germany and Russia) have been introduced into China since 1950s. These include Epoka (Poland), Mira and Aquila (East Germany) which have been grown predominantly in S.W. China. During this period about 200 cultivars have been released in China and of these, 93 superior cultivars have only six cultivars in their parentage (Table 1.3). This obviously means that the local cultivars have a too narrow genetic background (Qu and Chen, 1988). This clearly illustrates that in the first place, there is a lack of fully adapted cultivars, and secondly, even those that have been bred and selected as local cultivars have a narrow genetic base. It is imperative, therefore, for a more successful development of potato as crop, better strategies of crop improvement are required. In this context it should be emphasized that the cultivated potato has many wild relatives which also can contribute to the local improvement of this crop.

1.4 Potential of wild species and primitive cultivars

There are some 228 tuberous *Solanum* species recognized so far, and they are widely distributed in the Americas. They form a polyploid series based on the basic chromosome number of x=12 and there are triploids, tetraploids, pentaploids and hexaploids. Although there are polyploid forms, most of the wild species are diploids (Ochoa, 1990; Hawkes, 1992 and Human, 1994). Apart from the wild species there are cultivated species that are grown almost exclusively in the center of origin, Peru and Bolivia. These are *S. phureja, S. goniocalyx, S. ajanhuiri, S. stenotomum, S. chaucha and S. juzepczukii and S. tuberosum, subspp. andigena.*

Tuberous *Solanum* species have a wide geographical distribution and a very large range of ecological adaptations. In the Northern and Central Americas, they are found in Arizona, Colorado, New Mexico and Texas, Mexico, Guatemala, Honduras, Costa Rica and Western Panama, generally at medium to fairly high altitudes. In Southern America the wild species are found along the Andes from Venezuela through Colombia, Ecuador, Peru, Bolivia and Northwest Argentina. They also occur in the lowlands of Chile, Argentina, Uruguay, Paraguay and Southeast Brazil. Thus, some species are adapted to grow in the cold, very high Andean regions from 3000 to 4500 m, where frosts are very common (*S. acaule* and *S. megistacrolobum*). Whilst others occur in dry semi-desert conditions (e.g. *S. berthaultii S. tarijense and S. neocardenasii*), in cool temperate rain forest (e.g. *S. violaceimarmoratum* and *S. colombianum*), or some on the coastal plains of Argentina and surrounding countries (*S. commersonii* and *S. chacoense*).

There is, therefore, an extraordinarily wide range of habitats in which wild potatoes are found, and this underlines that they became adapted to several stress environments and developed strong resistances to a wide range of pests and diseases. On the other hand, cultivated potato has evolved under a very limited range of environmental conditions in cool temperate regions and is thus often less able to resist the attacks of pests and diseases occurring over the much wider range of conditions in which it is now cultivated. However, knowledge of the great ecogeographical range of wild species, contrasted with the originally very narrow range of cultivated ones, can assist in an understanding of the need for the use of wild species in potato breeding.

In view of the availability of more valuable sources of genetic variability in tuberous *Solanums*, there is great potential to adopt this crop more easily to new environments than it has been possible in the past. Besides many agronomic characters that can be introduced from wild species and primitive cultivated forms into the modern potato, there are numerous sources of disease resistance available.

Some of the wild species that can be important as sources of resistance for the major potato diseases are listed below.

1.4.1. Fungus resistance

Phytophthora infestan (late blight)
S. berthaultii, S. bulbocastanum, S. demissum, S. microdontum, S. phureja, S. pinnatisectum, S. polyadenium, S. stoloniferum, S. tarijense, S. tuberosum subspp. andigena, S. vernei, S. verrucosum.

Synchytrium endobioticum (wart)
S. tuberosum (both subspecies), also to R2 and R3 races in a range of wild species from Bolivia including *S. acaule, S. sparsipilum and S. spegazzinii* (from Argentina).

Streptomyces scabies (common scab)
S. chacoense, S. commersonii, S. yungasense and various cultivated species.

1.4.2. Bacterial resistance

Pseudomonas solanacearum (bacterial wilt)
Promising species are, in particular, *S. chacoense and S. sparsipilum.* Resistance is also found in *S. microdontum, S. phureja and S. stenotonum.*

Erwinia carotovora (softrot, blackleg)
Resistance found in some accessions of *S. bulbocastanum, S. chacoense, S. demissum, S. hjertingii, S. leptophyes, S. megistacrolobum, S. microdontum, S. phureja, S. pinnatisectum, S. tuberosum subspp. andigena* etc.

1.4.3. Virus resistance

Potato virus X (PVX)
S. acaule, S. chacoense, S. curtilobum, S. phureja, S. sparsipilum, S. sucrense, S. tarijense, S. tuberosum subspp. andigena.

Potato virus Y (PVY)
S. chacoense, S. demissum, S. phureja, S.stoloniferum, S. tuberosum subspp. andigena and S. brevidens.

Potato leaf roll virus (PLRV)
S. acaule, S. brevidens, S. etuberosum, S. raphanifolium.

Potato spindle tuber viroid (PSTV)
S. acaule from Peru (good resistance), *S. berthaultii and S. guerreroense*.

1.4.4. Insect resistance

Leptinotarsa decemlineata (Colorado beetle)
S. berthaultii, S. chacoense, S. commersonii, S. demissum, S. polyadenium, S. tarijense.

Myzus persicae, Macrosiphum euphorbiae (aphids)
S. berthaultii, S. brevidens, S. bukasovii, S. bulbocastanum, S. chomatophilum, S. infundibuliforme, S. lignicaule, S. marinasense, S. medianse, S. multidissectum, S. stoloniferum.

1.4.5. Nematode resistance

Globodera rostochiensis, G. pallida (potato cyst nematode)
S. acaule, S. boliviense, S. bulbocastanum, S. capsicibaccatum, S. cardiophyllum, S. gourlayi, S. oplocense, S. sparsipilum, S. spegazzinii, S. sucrense and S. vernei.

Meloidogyne incognita, M. chitwoodi and M. hapla (root-knot nematode)
S. bulbocastanum, S. chacoense, S. curtilobum, S. microdontum, S. phureja, S. sparsipilum, S. stoloniferum and S. tuberosum subspp. andigena (Janssen, et al., 1995, unpublished data).

1.5 Scope of the research

Looking to the economic importance and the world wide distribution of the cultivated potato, the efforts needed for the genetic improvement of this crop are self-evident. The availability of abundant sources of valuable genes from the wild relatives offers enormous possibilities for the improvement of this crop through breeding. In the prosperous North, as a result of intensive breeding, considerable success has been achieved. So much, that potato is an inexpensive source of food and an attractive industrial raw material. On the other hand, in the less prosperous South, the yields of potato are far below that of the Northern temperate regions. Because of its high yielding potential, it is attractive to grow potato in the more populous South where there is a need for cheaper food. As it is, the European and American cultivars cannot be as successful in the Southern tropical and subtropical regions as they are in the North. There are several reasons for this : 1) tuber yields will be low when the long day cultivars are grown in the short day conditions; 2) higher temperature in the tropics can reduce yields; most importantly 3) the ravage of diseases and pests are generally more severe in the tropics than in the temperate regions.

The above problems can be solved only through the development of cultivars that are more suitable for tropical and subtropical regions. There are, however, difficulties to breed potato because it is a highly heterozygous, vegetatively propagated polyploid crop. The inheritance of characters in autotetraploid potato is complicated and therefore, breeding of this crop is difficult and time consuming.

1.6 The problem of polysomic inheritance

It is now well established that *S. tuberosum* is a tetraploid (2n=4x=48) of complex origin. Probably, an F1 hybrid between the two closely related diploid (2n=2x=24) species, *S. stenotomum* and *S. sparsipilum*, gave rise to the tetraploid after chromosome doubling. Subsequently, because of extensive hybridization with the wild relatives in its natural habitat as well as in the process of crop breeding, the genome composition of the potato was drastically altered. Besides the complicated origin, this crop is vegetatively propagated and has retained a high degree of heterozygosity. As may be expected, because of its complex origin, its tetraploid constitution and the high degree of heterozygosity, the inheritance of characters in potato is highly unpredictable. This, obviously, complicates the breeding procedures.

One method of simplifying potato breeding is to produce diploid forms of cultivated potato, the so-called dihaploid (2n=2x=24), and to breed them at the diploid level. The use of diploid cultivated potato in breeding was first suggested by Chase (1963), the so-called "analytic breeding". Fortunately, dihaploids of potato can be produced from the cultivars routinely in relatively large numbers (Hermsen and Verdenius, 1973). The advantages of breeding potato at the diploid level are: 1) undesirable recessive alleles can be eliminated; 2) Mendelian inheritance will be more straightforward and, therefore, the selection for desirable characters can be more convenient; 3) dihaploids can be easily hybridized with the large number of diploid wild species to introduce valuable genes from the latter. Because of these advantages, potato breeding at the diploid level is becoming increasingly popular.

Once the diploids are improved through breeding, they can be used to produce the tetraploid cultivars. For this purpose, the selected dihaploids are sexually polyploidized through the use of numerically unreduced gametes (2n-gametes). This implies that the improved diploids must be selected in such a way that, besides desirable agronomic traits, they also produce 2n-gametes.

In the past, research on potato at the University of Wisconsin (USA) and the Department of Plant Breeding at the Wageningen Agricultural University in the Netherlands, has opened the possibilities for producing dihaploids on a large scale and for selecting diploid genotypes that produce sufficient frequencies of 2n-gametes

(Hougas and Peloquin, 1964; Hermsen and Verdenius, 1973; Mendiburu and Peloquin, 1971; Mok and Peloquin, 1975; Ramanna, 1979, 1983; Jacobsen, 1980; Jongedijk, 1991; Jacobsen et al., 1991, among others). Dihaploids are produced through the so-called "prickle pollination" of potato styles with the pollen of *S. phureja*, a diploid cultivated species. With this method, the occasional parthenogenetically developed seeds can be selected out with help of the dominant genetic marker "embryo-spot". Thus, the parthenogenetically developed seeds without embryo spot give rise to dihaploids (Hermsen and Verdenius, 1973).

With respect to 2n-gametes, there are genetically two different types produced. These are the so-called first division restitution (FDR) and second division restitution (SDR) gametes. FDR gametes originate through one equational division of the whole chromosome compliment during second meiotic division. Genetic recombination is restricted to crossing-over during pairing of homologous chromosomes. Because of this, all FDR gametes can be genetically similar, and parental gene combinations remain mainly intact. They are favourable for transferring heterosis from the diploid parent to the progeny. SDR gametes, on the other hand, originate through endomitosis of the products of meiotic reduction. This means, they will be highly dissimilar to each other (heterogeneous), homozygous and split up the parental gene combinations both by random chromosome assortment and crossing-over. Both types of 2n-gametes can be useful in different situations, but FDR gametes are most pertinent in analytic breeding and TPS production (see later).

The selected diploid clones which produce 2n-gametes can be used for inducing sexual polyploids in two ways: a) unilateral sexual polyploidization and b) bilateral sexual polyploidization. If only one of the parents contributes the 2n-gametes to produce a polyploid progeny in 4x-2x or 2x-4x crosses, it is called unilateral. If 2n-gametes from both parents have contributed to the polyploid progeny in 2x-2x crosses, it is bilateral. As a final step in analytic breeding, both of the methods can be useful. However, for practical breeding 4x-2x crosses may be more attractive.

1.7 Production of true potato seed progenitors

Because of the vegetative propagation of potato, it is almost impossible to avoid infections with viruses. Such diseases, besides other causes, are responsible for the so-called seed degeneration. This means that once the tubers are multiplied in a tropical area, they can no longer be used as planting material because the yield of tubers will be highly reduced. To avoid this, as a rule, the planting material grown in a cooler area has to be imported time and again. Such imported seed tubers are prohibitively expensive and a risky investment for the small farmers. One solution to this problem is to raise the potato crop through sowing true botanical seed. There are many advantages in this method: a) diseases that are not transmitted through seeds, such as virus diseases, can be prevented; b) seed material is quite inexpensive and the small farmers can easily afford it; c) the technology does not require highly skilled labour or investments and the farmer can produce seed material on his own if necessary.

There are, however, problems to produce TPS cultivars. First, as was already pointed out, the potato is a highly heterozygous and vegetatively propagated crop. Generally, the seeds produced either by selfing or crossing of tetraploid cultivars give rise to highly heterogeneous progenies genetically and phenotypically. This can be a limitation when highly uniform yields and other tuber characters are desired. Secondly, because of vegetative propagation, potato cultivars can be highly sterile and harbour many deleterious recessive genes. These can limit the production of hybrid seeds and seedlings on a large scale due to inbreeding depression.

The problem of heterogeneity of the tetraploid potato can be resolved to some extent through the use of diploids. For example, the diploids can be crossed with diploid primitive cultivars of the species such as *S. phureja* or *S. stenotomum* and the progeny be selected for agronomic characters. The desirable genotypes which also produce high frequencies of 2n-gametes, especially 2n-pollen through FDR, can be selected and used as parent in TPS varieties via 4x-2x crosses. Because FDR can partly preserve the heterozygosity and favourable intralocus and interlocus interactions of the selected diploid genotypes, these can be directly used as male parent in TPS varieties based on 4x-2x crosses. Because the 4x female can offer horticultural traits and FDR 2n-pollen contribute uniformity, resistance and other counterpart traits in the 4x-2x crosses, the resulting tetraploid progenies of such crosses are expected to be frequently more vigorous, more uniform and agronomically more acceptable not only phenotypically but also genetically than 4x-4x progenies. Thus, the outlined procedure meets the essential requirements for developing TPS cultivars.

1.8 The aim of this research

Based on the available basic knowledge of the 2n-gametes in potato, it was proposed to develop systematic methods of using 2n-pollen in analytic breeding and in TPS cultivar development suitable for tropical and sub tropical regions. In this context, the experimental work was directed to answer the following specific questions:

1). What is the genetic basis of 2n-pollen occurrence?

2). What are the strategies for selecting diploid clones that produce high frequencies of 2n-pollen?

3). How effective are the 2n-pollen producing genotypes for breeding and selecting of agronomically desirable traits in the progenies like resistance to bacterial wilt?

4). Is it possible to attain the required vigour, uniformity and agronomic traits in the sexual polyploid progeny of 4x-2x crosses more frequently than 4x-4x crosses expressed in the seedling and/or clonal generation for the development of TPS cultivars?

In compiling of this thesis, Chapter 2 describes the results on the quantitative estimates of 2n-pollen formation in the progenies of diallel crosses among five well-known diploid potato clones. In addition, heritabilities of 2n-pollen formation in broad and narrow sense are estimated. In Chapter 3, estimates of the frequencies of 2n-pollen in different types of populations of crosses involving low (low frequency of 2n-pollen) x low, low x high, high x high and high x high-backcrosses, are presented. Based on these results, an insight has been gained into the methods of increasing the frequencies of 2n-pollen during selection. In Chapter 4, in order to test the effectiveness of 2n-pollen genotypes in analytic breeding, a quantitative character that controls the resistance against bacterial wilt, caused by *Pseudomonas solanacearum*, has been transferred through 2n-pollen into the 4x progenies. Finally, in Chapter 5, the possibilities for TPS production and the scope for analytic breeding through the use of 4x-2x and 4x-4x crosses have been assessed. In the case of the progenies of 4x-2x crosses about 15 characters including four agronomic traits (tuber number, tuber yield, marketable rate and specific gravity) have been evaluated in the seedling and/or clonal generation and the implications for TPS breeding are discussed.

Table 1.1 Share (%) of potato production in developing countries by agroecology (A) and by continental region (B).*

A.

	Latin America	N. Africa W. Asia	Asia	sub Saharan	Total %
Semi arid tropics	73.9	0.0	0.0	26.1	100
Humid tropics	6.1	0.0	90.7	3.2	100
Subtropical lowlands	3.0	0.0	97.0	0.0	100
Arid and Mediterranean	15.5	54.7	29.6	0.2	100
Highlands	55.6	15.6	12.5	16.3	100
Temperate	10.0	17.0	73.0	0.0	100

B.

	sub-Saharan Africa	Asia W.Asia	N.Africa America	Latin America	Total %
Semiarid tropics	5.3	0.0	0.0	2.9	0.7
Humid Tropics	0.9	1.3	0.0	0.3	0.9
Subtropical lowlands	0.0	58.6	0.0	6.8	38.7
Arid and Mediterranean	0.7	6.7	52.0	13.2	14.6
Highlands	93.1	3.7	19.2	61.5	19.0
Temperate	0.0	29.7	28.8	15.3	26.1
	100	100	100	100	100

* (Adopted from CIP publication 1993: programme report).

Table 1.2 Some of the older cultivars still in cultivation in their native countries and China (Qu, et al., 1988).

Cultivar	Year released	Country still growing
Russet Burbank	1876	U.S.A
May Queen	1900	Britain
Bintje	1910	Netherlands
Irish Cobbler	1878	U.S.A, Japan
Chippenwa	1933	U.S.A
Early Rose	1861	U.S.A
Triumph	1878	U.S.A
Houma	1936	U.S.A
Katahdin	1932	U.S.A
Mira	1952	Germany, China
Epoka	1955	Poland, China
292 (origin: Houma)	1944	China

Table 1.3 The genetic background of major cultivars released in China since 1950s (Qu, et al., 1988).

Parents	Cultivars involved in parentage		Examples of Chinese cultivars
	Number	Percentage	
292-20 (Houma)	23	25	Kexin series
Katahdin	14	15	BS series, Tigerhead
Epoka	14	15	Heap
Mira	8	8.5	Xinyu 4
Anemome	6	6.5	NEA303, early cultivars
Local variety	7	7.5	Bashu series

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Chapter 2

A comparison of progeny from diallel crosses of diploid potato with regard to the frequencies of 2n-pollen

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(Submitted)

Summary

Selection of diploid potato genotypes that produce high frequencies of 2n-pollen in different environments is an important requirement for analytic breeding as well as for true potato seed production. In this context, quantitative estimates of 2n-pollen formation were made in two types of diploid potato populations. One population set consisted of the progeny from diallel crossings of five well-known diploid potato clones (A=USW5293-3, B=USW5295-7, C=USW5337-3, D=USW7589-2 and E=77-2102-37). Another population set was obtained by pollinating three genotypes of *Solanum phureja*, IvP35, IvP48 and IvP101 with clones A, C and E. It was observed that the behaviour of the parental clones with respect to 2n-pollen formation in different environments, such as in Europe and China, was rather stable. The coefficient of correlation between means of 2n-pollen frequency of combinations and percentage of genotypes with more than 5% of 2n-pollen in a given combination was estimated as also the coefficient of correlation between mean and maximum frequency of 2n-pollen formation in each combination. The results showed that the former coefficients were significant and the latter were not. The combining ability analysis indicated variation between families. Variation in specific combining ability (SCA) effects contributed much more than general combining ability (GCA) variation to variation in 2n-pollen formation. The broad-sense heritability of 2n-pollen formation was estimated to vary between 0.45 and 0.53 and the narrow-sense heritability was calculated to be 0.15 and 0.20 in the two types of populations. This means that additive variance was relatively low.

Key words: combining ability, diallel cross, diploid potato, heritability, unreduced gametes

Introduction

Numerically unreduced gametes (2n-gametes) are very useful in the breeding of polysomic polyploid crops such as potato, alfalfa and red clover among others (Peloquin, 1983; Bingham, 1980; Parrott and Smith, 1986; Mariani and Tavolatti, 1992). In these crops, 2n-gametes have been used for inducing polyploids through the so-called process of sexual polyploidization. This process is helpful for increasing crop yields through maximizing heterozygosity (Mendoza and Haynes, 1973; Bingham, 1980; Watanabe, 1991; Hutten, 1994) as well as for the enhancement of germplasm (Peloquin et al., 1989; Qu et al., 1988, 1993).

In the cultivated potato, *Solanum tuberosum* (2n =4x=48), the use of 2n-gametes was first proposed by Chase (1963) who suggested a breeding method called "analytic breeding". According to this method, tetraploid cultivars are reduced to diploid level (2n=2x=24). These "dihaploids" are used for breeding potato at the diploid level using diploid wild species. From the improved diploid clones tetraploid cultivars are produced through sexual polyploidization. In this context efficient methods have been developed for the production of potato dihaploids (Hermsen and Verdenius, 1973), and diploid potato clones that produce 2n-gametes have been selected in the past (Mendiburu and Peloquin, 1977; Jacobsen, 1980).

Among many of the 2n-gamete forming diploid potato clones, the following five genotypes have been widely used in breeding programmes as well as in cytogenetic investigations: USW5293-3, USW5295-7, USW5337-3, USW7589-2 and 77-2102-37. In some of these clones simple recessive genes that control 2n-gamete formation were reported (Mok and Peloquin, 1975a; 1975b) and the cytological modes of their origin were studied (Ramanna, 1979; 1983; Jongedijk and Ramanna, 1988). Although the cytological abnormalities that lead to 2n-gamete formation in these clones are reported to be controlled by single recessive genes, these have been found to be major genes which are highly influenced by the environment (Jacobsen, 1980; Ortiz and Peloquin, 1992; Veilleux et al., 1982).

If 2n-gametes have to be exploited more efficiently in potato breeding, there are three important prerequisites: a) they should be produced in high frequencies; b) the genotype should be stable with respect to 2n-gamete formation; and c) the mode of their origin should be known. In order to pave the way for the selection of such clones, the five above mentioned diploid potato clones were crossed in a half diallel combination and the progeny was evaluated for the frequencies of 2n-pollen formation. Besides these, the progeny from crosses between three clones of *S. phureja* and three of the above mentioned clones, A, C and E, were used for comparison. These results form the subject of this article.

2.1. Material and methods

2.1.1 Material

Eight diploid potato clones ($2n=2x=24$) which were selected at the University of Wisconsin (USA), the Max-Planck Institute, Cologne, Germany and at the Department of Plant Breeding (IvP), Wageningen, the Netherlands were used. Among these, five clones were originally derived from crosses between dihaploids of *Solanum tuberosum* and the diploid species *S. phureja* (for details of the pedigree, see Jongedijk and Ramanna, 1988). These five clones were: USW5293-3 (coded as A), USW5295-7 (B), USW5337-3 (C), USW7589-2 (D) and 77-2102-37 (E). The other three clones, IvP35, IvP48, and IvP101, were selected originally from *S. phureja* as pollinator for parthenogenetic dihaploid production through 4x-2x crosses. All these eight clones have been used world-wide (i.e., USA, Canada, Mexico, Peru, Chile, Argentina, Italy, France, Germany, the Netherlands, UK and China) in potato research. Besides these clones, a number of other diploid genotypes were used for evaluation of 2n-pollen formation in the Netherlands and China.

2.1.2 Methods

Two types of crosses were made in 1992 by using plants grown in greenhouses at the Department of Plant Breeding, Wageningen: 1) diallel crosses involving clones A, B, C, D and E; and 2) three IvP clones, which produced a very low frequency or no 2n-pollen, were pollinated by clones A, C and E.

The seeds resulting from these crosses were used for growing progeny in China during 1993. About 100-200 seeds per combination were soaked at 30^0 C in a solution of 0.2% potassium nitrate (KNO_3) for 24 hours and then incubated at 25^0 C in water for five days, each day rinsing with fresh water. Subsequently, these seeds were sown in seed beds made of a mixture of sandy soil and peat with a 2-3 cm top layer of that mixture substrate. Moisture and temperature of the seed bed was regulated by covering with a plastic tunnel. Four weeks later the plastic tunnel was removed in order to acclimatize the seedlings to field conditions and allowed them for another two weeks. These seedlings were transplanted into the field with a spacing of 40 x 60 cm during early June. The tuber families (one tuber from each plant was assorted) collected from the seedling generation in 1993 were grown in early May, 1994. The experimental field was located at the Research Station for Highland Agriculture in Bashang, Hebei province, China at 41^0 N with an altitude of 1,500 m.

The plants flowered during July. Pollen was collected from fully opened flowers, mounted in a 2% solution of acetocarmine on a microscopic slide; the pollen size and the number of pollen germpores were monitored. The classification of pollen into n- and 2n- was according to Ramanna (1979) and Jacobsen (1980) where a diameter of less than 23 mμ with three germpores was scored as n-pollen and larger than this size with four germpores as 2n-pollen.

Frequencies of n- and 2n-pollen were estimated. Ten out of 18 combinations from diallel crosses of A, B, C, D and E clones with four replicates of 10 plants of each were evaluated according to the fixed model of Griffing's Method IV (Liu, et al., 1984). The other derived from crossing IvP35, IvP48 and IvP101 with clones A, C and E formed the so-called primary population. The statistics were based on the model of incomplete diallel crosses. The number of genes determining the 2n-pollen expression was estimated using the approximate formula, $n=R^2/(8\ \delta^2_A)$ (Falconer 1981), where R is the total range of response to selection, in this case being 0 to 1, with 1 (100% 2n-pollen frequency) being a theoretical maximum. Narrow-sense heritability (h^2_N) was estimated as a ratio of the additive genetic variance (δ^2_A) to the phenotypic variance. Broad-sense heritability (h^2_B) was estimated as a ratio of the total genetic variance to the phenotypic variance.

2.2. Results

2.2.1 Stability of 2n-pollen formation in diploid clones

For the classification of plants as n- and 2n-pollen producers, the pollen size and number of germpores were quite clear criteria in these materials. In most cases, it was possible to classify 2n-pollen based on their larger size as compared to the n-pollen grains. As a first step, all 19 diploid parental clones were examined for the presence or absence of 2n-pollen. A notable feature was that their behaviour with respect to 2n-pollen formation was highly correlated across three locations even though when the plants were grown in different environments (Table 2.1). For example, clones A and D produced relatively low frequencies (5% or less) whereas clones B, C and E produced more than 5% 2n-pollen in three locations. So also, the IvP clones produced very low frequencies or no 2n-pollen in all the four places. As in the case of the parental clones, some of the hybrid lines, e.g., CD1045 (line from C x D), CE1063 and ED1022 produced high frequencies of 2n-pollen in different environments. Thus the performance of these clones was relatively stable.

Besides the occurrence of 2n-pollen, the stainability of pollen in general was an important consideration because it was an indication of the level of fertility. In most of the clones the pollen stainability exceeded 50% (Table 2.1) and therefore they were potentially useful as male parents.

2.2.2 Frequencies of 2n-pollen formation in sexual offspring

The frequencies of 2n-pollen were estimated in two types of populations. One population set was derived from crossing the IvP clones, which produced either very low frequencies or no 2n-pollen, with clones A, C, and E. These formed the so-called "Primary population" and the mean percentages of 2n-pollen across 40 randomly selected plants from four replicates for each combination are presented in Table 2.2.

Unlike the above population, another population set was derived from a diallel crossing of the five clones, A, B, C, D and E. These clones, as different from the IvP clones, were previously selected for 2n-pollen production (see material and methods). Because of this, the progenies of these clones were indicated as "Secondary population". The mean percentages of 2n-pollen estimated across 40 randomly selected plants, as described for the primary population, are given in Table 2.3.

The sexual progenies included in Table 2.2 and 2.3 can be roughly classified into three categories. There were the progenies resulting from crosses in which a) both parents produced either a low frequency (less than 5%) or no 2n-pollen (e.g., IvP35 x A, IvP48 x A, IvP101 x A and A x D); b) only one of the parents produced high frequency of 2n-pollen (e.g., IvP35 x C, IvP35 x E, IvP48 x C, IvP48 x E, IvP101 x C and IvP101 x E ; A x B, A x C, A x E, C x D and D x E); c) both parents produced high frequencies of 2n-pollen (e.g., B x C, B x E and C x E).

There was a clear variation for the frequencies of 2n-pollen between the combinations (Tables 2.2 and 2.3). Based on the criterion of mean of the percentages, the highest frequency was observed in the combination A x B (12.88% in Table 2.3) in which one of the parents (A) was a low 2n-pollen producer. This was significantly different from other similar combinations as well as from the progeny of parents both of which produced high frequencies (e.g., B x E). This means that a clear pattern was lacking with respect to the performance of the progeny; in other words, the outcome was clearly not predictable.

A comparison of the overall means of the primary and secondary population indicated that there was a notable increase in the frequencies of 2n-pollen in the latter (5.24 and 8.06, Tables 2.2 and 2.3 respectively). The genotypes that produced more than 5% of 2n-pollen also varied considerably across combinations. The highest frequencies of plants with more than 5% 2n-pollen was found in the A x E combination (69.8%), followed by B x C, A x B and D x E with more than 50% (Table 2.3).

In the primary population IvP35 x E gave the highest 2n-pollen frequency (8.34%), which was 4.54% lower than the highest combination of the secondary population (A x B =12.88%). IvP35 x E was also significantly different from those combinations with less than 5.51% (e. g., IvP101 x A). IvP48 x A produced the lowest frequency of 2n-pollen (1.36%) (Table 2.2). The coefficient of correlation between the percentage of plants with more than 5% 2n-pollen and mean of 2n-pollen frequency in a combination was found to be r=0.89 and significant at p=0.01 level. In the secondary population, the coefficient of correlation between the percentage of plants with more than 5% of 2n-pollen and combination means indicated r= +0.88 which is a significant difference at p=0.05 level. The coefficient of correlation between the mean of 2n-pollen and maximum 2n-pollen frequency in a given combination r=0.15 amounted to the primary population and to r=0.46 for the secondary population (Table 2.2 and Table 2.3). Meanwhile the coefficient of correlation between maximum 2n-pollen frequency and the percentage of plants with more than 5% 2n-pollen were estimated at 0.12 for the primary population and 0.35 for the secondary population and both were not significantly different (Table 2.2 and Table 2.3).

2.2.3 Analysis of combining ability of 2n-pollen formation

The combining ability analysis was carried out for both the primary and the secondary population. In the primary population, IvP35 and IvP101 had a similar GCA, with 0.68 and 0.69 respectively, whereas IvP48 had a negative value of -1.37 (Table 2.2). So also, there was a clear difference between clones A, C and E with respect to GCA (Table 2.2) within the primary population in which the clone A showed a negative value (-1.56) whereas C and E showed positive values of 0.58 and 0.98 respectively.

In the secondary population there were also clear differences in GCA values between the genotypes (Table 2.3). The highest positive value was observed for clone A (+3.54) and the lowest negative value was found for clone D (-1.55). Clones B, C and E had more or less similar GCA values for 2n-pollen formation. A notable feature was that clone A showed a negative value (-1.56) in the primary population, whereas a positive value (+3.54) was highly evident in the secondary population.

In general, GCA was an indication for additive gene effects and SCA was a reflection of non-additive gene effects. In the primary population SCA was the highest for the combination of IvP48 x C (2.78). It was that the combination produced the highest mean percentage of 2n-pollen (IvP35 x E) differed from the combination with the highest SCA value (IvP48 x C) (Table 2.2). In secondary population SCA varied from +2.28 (B x C) to -2.37 (B x E). For other combinations, D x E, A x E, A x B and C x D there were positive values in a decreasing order (Table 2.3). The overall conclusion from the analyses of GCA and SCA was that the evaluation of only GCA was insufficient. However, from the point of view of selection for high frequencies of 2n-pollen formation, SCA of each combination might be more helpful. Based on these analyses of all the clones, it appeared that E and IvP35 were probably the best parents followed by C and IvP101 (Table 2.2 and 2.3).

2.2.4 Genetic control of 2n-pollen formation

Broadly, the phenotypic behaviour of different parental clones in diverse environments was according to prediction. For example, all the parental clones (Table 2.1) were tested over the years for 2n-pollen production at the Department of Plant Breeding (Wageningen). It was found that some of the clones produced a low frequency of 2n-pollen (e.g., IvP35, IvP48, IvP101, A and D) whereas others had relatively high frequencies of 2n-pollen (e.g., B, C, E, CD1045, CE1063, ED1022 and CE10). This phenotypic behaviour persisted when these clones were grown in different environments in China (IVF, Bashang and Hainan). This means, despite environmental effects, that the influence of a genotype on the frequencies of 2n-pollen was evident. It was, therefore, an indication that 2n-pollen formation was genetically controlled. From a comparison of additive variance, non-additive variance, genotypic and phenotypic variance (Table 2.4), it was evident that the primary population showed a relatively limited variation as compared to the secondary population. The estimate of broad-sense heritability in the primary population was 0.53 and the narrow-sense heritability was 0.15. For the secondary population these were 0.45 and 0.20 for broad- and narrow-sense heritability respectively (Table 2.4). This suggested that genetic differences existed among the parents of the primary population so that non-additive variance contributed more than the additive variance. There were, however, relatively small differences between the parents in the secondary population. Consequently, the non-additive effect was relatively less conspicuous than the additive variance. This was probably due to the increment of the number genes that influenced the occurrence of 2n-pollen.

The number of genes that determine 2n-pollen formation in the two sets of populations were estimated according to the formula, $n= R^2/(8\ \delta^2_A)$ (Falconer, 1981). The results suggested that in the primary population two genes (n=1.55) and in the secondary population probably four genes (n=3.75) were involved. From the combining ability estimates in two populations, it was evident that the SCA variation was predominant with 97.05% of variance in the primary population, whereas the GCA variation amounted to 28.43% in the secondary population, as compared to 2.95% in the primary population.

2.3. Discussion

All diploid potato clones used in this study have been previously investigated for the cytological modes of origin of 2n-pollen (Mok and Peloquin, 1975a, 1975b;

Ramanna, 1983; Jongedijk and Ramanna, 1988; Hutten, 1994). From these investigations it is clear that there are several cytological mechanisms existing within these clones. A remarkable feature is that some of the cytological abnormalities that lead to 2n-gamete formation, such as "parallel spindle" (ps), "precocious cytokinesis" (pc) were shown to be controlled by a single recessive gene (Mok and Peloquin, 1975a; 1975b). If there were such clear-cut single recessive genes, the task of introducing 2n-pollen formation into the desirable clones would be straight forward. Unfortunately, the cytological mechanisms that give rise to 2n-pollen are not that simple (Ramanna, 1979), nor is their genetic control straight forward (Veilleux and Lauer, 1981; Jacobsen, 1976; Ramanna, 1983). Nevertheless, there are indications that the 2n-pollen formation in potato is genetically controlled, probably by both major and minor genes, whose actions are influenced by the environment (Veilleux et al., 1982, Ramanna, 1979; Jacobsen, 1976).

The complicated genetic control of 2n-pollen formation in potato is evident from the fact that a clear-cut classification into distinct phenotypic classes is not possible in a segregating population. For example, Mok and Peloquin (1975a and 1975b), using a criterion of 5% 2n-pollen producers as recessive homozygotes (ps/ps or pc1/pc1) found a clear cut monogenic Mendelian segregation. Later, however, 1% 2n-pollen was used as a criterion to score for a similar segregation (Iwanaga and Peloquin, 1984), which was once again inadequate to explain the segregation (Hermsen et al., 1985; Bani-Aameur et al., 1992). In view of these difficulties, the present approach of quantifying the frequencies of 2n-pollen in the progenies of diallel crosses might be useful for the elucidation of the genetic control of 2n-pollen formation as well as for selecting desirable clones.

Generally, the genotypes that produce high frequencies of 2n-pollen are considered to be good parents and used for the production of progenies with improved performance for the trait (Ortiz and Peloquin, 1992; Barone et al., 1993). However, in these investigations only the progenies that produced higher frequencies of 2n-pollen were taken into account, but not the percentage of superior genotypes that occurred in a particular combination. If the aim is to combine several agronomic characters with 2n-pollen production, it is important to use superior parents that give rise to a higher percentage of genotypes in their progeny that produce high frequencies of 2n-pollen. In this context, the clones A, IvP35 and IvP48 provide good examples. Although these clones produce relatively low or no 2n-pollen (Table 2.1), they are good parents in certain combinations such as A x B, IvP35 x E and IvP48 x C (Table 2.2 and 2.3). On the contrary, some of the parents that produce high frequencies of 2n-pollen (e.g., B, C and E) give rise to progenies that produce low mean percentages of 2n-pollen (e.g., B x E; C x E, Table 2.3).

The genetic control of 2n-pollen formation is much more complicated than originally reported by Mok and Peloquin (1975a and b). From the results of the present investigation, as in previous studies (Jacobsen, 1976; Veilleux et al., 1982), the inheritance of 2n-pollen formation in potato is quantitative rather than qualitative. It is also evident that on the one hand, the environmental conditions greatly influences the occurrence of 2n-pollen, on the other hand, such selected 2n-pollen producers can be used in different climates as significant correlation coefficients of 2n-pollen production between three locations indicated (Table 2.1). Besides these two constraints, for a critical study of the genetic control of 2n-pollen formation, it should be pointed out that potato is highly heterozygous and that a distorted Mendelian segregation is common. Despite such constraints, the approximate number of genes involved in 2n-gamete formation has been estimated in potato as well as other crops (Ortiz and Peloquin, 1992; Parrott and Smith, 1986). In the present investigation the number of gene involved in 2n-pollen formation appears to be 2-4 rather than a single gene.

The genetic parameters estimated (Table 2.4) suggest that the heritability of 2n-pollen formation in the populations was not high. However, there are indications that the additive genetic variance is increased in the secondary population. This means that recurrent selection can be effective. In potato, a vegetatively propagated crop, the non-additive effects can be fixed and additive effects be accumulated by recurrent selection. The selection response may increase quickly by adopting these two kinds of genetic effects and such clones can probably be utilized in breeding programmes in the different regions.

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Table 2.1. 2n-pollen frequency (%) and pollen stainability of different genotypes at IvP, Wageningen Agricultural University and in different environments (IVF/Beijing, Bashang/Hebei and Hainan) in China.

Genotype	IvP (greenhouse)		IVF		Bashang		Hainan	
	2n-pollen	STN	2n-pollen	STN	2n-pollen	STN	2n-pollen	STN
A	5	25	1	5	0	0	–	–
B	47	83	20	54	30	75	–	–
C	15	46	15	63	8	40	–	–
D	4	95	5	65	2	60	–	–
E	23	68	10	77	20	80	–	–
IvP35	0	90	0	30	4	78	2	95
IvP48	0	95	0	65	0	90	1	90
IvP101	2	20	0	30	0	46	–	–
CD1045	35	94	20	80	12	88	24	90
ED1045	15	30	9	20	–	–	–	–
EC394	10	86	8	60	13	76	–	–
CE1063	36	30	10	10	25	46	18	60
ED1022	30	50	15	41	20	60	–	–
CD1047	10	30	5	50	4	33	14	85
CE10	40	45	26	30	13	15	23	28
CE13	–	–	1	5	17	58	14	30
Q87350-3	20	30	5	10	30	70	–	–
CD1015	20	85	8	58	9	72	–	–
EC373	15	15	6	87	–	–	3	92

Correlation coefficient of data from three locations:

2n-pollen	IvP and IVF	r=0.88**
	IvP and Bashang	r=0.77**
	IVF and Bashang	r=0.52*
STN	IvP and IVF	r=0.49*
	IvP and Bashang	r=0.74**
	IVF and Bashang	r=0.58*

IvP: all materials were grown in the greenhouse where it is cool during summer.
IVF: material was grown in spring and fall with hot summer (30-35°C).
Bashang: a single cropping area with cool and dry summer and high altitude (1,500m).
Hainan: a typical tropical and winter cropping season for potato with latitude (18°N).
STN: Percentage of stainable pollen (n+2n). – Data could not be collected.
* Significant difference from zero at p=0.05 level;
*** Significant difference from zero at p=0.01 level.

Table 2.2. Frequencies of 2n-pollen and combining ability analysis in the primary population from *Solanum phureja* x A, C and E clones (Ten random plants for each of the four replicates).

	2n pollen formation			SCA	GCA
Pedigree	Mean % per* combination	Maxi. %	% of plants with > 5% 2n pollen		
IvP 35 x A	4.69 bc	28.6	34.3	0.33	IvP 35=0.68
IvP 35 x C	4.73 bc	24.0	33.3	-1.77	IvP101=0.69
IvP 35 x E	8.34 a	24.0	45.5	1.45	IvP48=-1.37
IvP 101 x A	5.01 bc	16.7	31.7	0.63	A=-1.56
IvP 101 x C	5.51 abc	33.3	45.0	-1.01	C=0.58
IvP 101 x E	7.30 ab	75.0	41.1	0.38	E=0.98
IvP 48 x A	1.36 d	27.3	14.1	-0.95	
IvP 48 x C	7.24 ab	27.3	52.4	2.78	
IvP 48 x E	3.02 cd	47.7	30.7	-1.83	
Grand mean	5.24	33.8	36.5		
Correlation		+0.15	+0.89**		
Coefficient (r)			+0.12		

* Duncan's significant difference test at p=0.05 level.

** Coefficient of correlation significant difference from zero at p=0.01.

Table 2.3. Frequencies of 2n-pollen and combining ability analysis in the secondary population (Ten random plants for each of the four replicates).

	2n pollen formation			SCA	GCA
Pedigree	Mean % per* combination	Maxi. %	% of plants with >5% 2n pollen		
A x B	12.88 a	46.2	55.9	1.77	A= 3.54
A x C	8.69 abc	57.7	46.0	-2.12	
A x D	8.52 abc	30.8	45.7	-1.51	
A x E	12.77 ab	51.3	69.8	1.86	
B x C	9.02 abc	28.8	58.0	2.28	B=-0.53
B x D	4.29 c	46.3	37.	-1.68	
B x E	4.47 c	26.3	32.6	-2.37	
C x D	6.97 c	17.4	45.3	1.25	C=-0.78
C x E	5.17 c	30.0	40.2	-1.42	D=-1.55
D x E	7.77 bc	35.3	53.1	1.94	E=-0.67
Grand mean	8.06	37.0	48.5		
Correlation		+0.46	+0.88**		
Coefficient(r)		+0.35			

* Duncan's significant difference test at p=0.05 level.

** Coefficient of correlation significant difference from zero at p=0.01 level.

Table 2.4. Estimate of genetic parameters in the two populations.

Genetic parameters	Primary population	Secondary population
Additive variance	1.1320	3.5468
Non-additive variance	2.7946	4.4638
Genotypic variance	3.9266	8.0106
Phenotypic variance	7.6000	17.6654
Broad-sense heritability(%)	52.66	45.35
Narrow-sense heritability(%)	14.89	20.08
Genetic variation due to GCA(%)	2.95	28.43
Genetic variation due to SCA (%)	97.05	71.57

Chapter 3

Repeated cycles of selection for increasing the frequencies of 2n-pollen in diploid potato populations

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Summary

Some of the well-known diploid potato clones that produced different frequencies of 2n-pollen were intercrossed in the following types of combination: low x low (low frequency of 2n-pollen), low x high, high x high and high x high-backcross. In these progeny populations the frequencies of 2n-pollen were evaluated using 3,971 plants from 36 combinations. Although there was an indication that 2n-pollen formation was genetically controlled, the pattern of inheritance was not monogenic. A quantitative assessment of 2n-pollen formation in different populations indicated that there was a great variation between the progenies of different combinations. Thus, the population mean percentage of 2n-pollen producing plants in high x high-backcross combinations (10.7%) was fourfold higher as compared to that of low x low combinations (2.4%). Because plants with high frequencies of 2n-pollen were selected from the low x low combinations, it was evident that the alleles inducing 2n-pollen formation were already present but not clearly expressed in these parents. Through an appropriate repeated selection of parents, the percentage of plants with 2n-pollen production and with higher frequencies of 2n-pollen could be produced effectively in the populations. The mode of origin of 2n-pollen in these populations was predominantly through FDR. These genotypes can be useful in analytic breeding as well as for true potato seed production.

Key words: *Solanum tuberosum*, unreduced pollen, true potato seed, analytic breeding.

Introduction

Generally, it is difficult to breed polysomic polyploids (autoploids) because the Mendelian segregations are more complicated. As a result, the selection of a combination of desirable characters in their progenies is much more laborious as compared to breeding at the diploid level. During the last two decades, however, considerable progress in ploidy manipulation has been made in certain polysomic polyploids such as alfalfa (*Medicago sativa*), Red Clover (*Trifolium pratense*) and potato (*Solanum tuberosum*) (McCoy, 1986; Parrott et al., 1986; Watanabe and Peloquin, 1991; Ramanna and Jacobsen, 1992). Especially in *S. tuberosum* (2n=4x=48), following the suggestion of Chase (1963), breeding at the diploid level (2n=2x=24) and returning to the tetraploid condition has become quite attractive (Qu, et al., 1988 a,b; De Jong and Tai, 1991; Werner and Peloquin, 1991; Hutten, 1994; Clulow et al., 1995). One of the crucial steps in this procedure of "analytic breeding" (Chase, 1963), is the induction of tetraploids through the use of 2n-gametes.

There are many advantages of breeding potato at the diploid level and using 2n-gametes to produce tetraploidized plants. First of all, unlike colchicine doubling which leads to inbreeding depression (Watanabe et al, 1991), through the use of 2n-gametes heterozygosity can be maximized in the tetraploid progeny (Mendiburu and Peloquin, 1974; Bingham, 1980; Peloquin 1992; Tai, 1992). Secondly, it is possible to produce highly vigorous and relatively uniform 4x-progeny even when the diploid parents are highly heterozygous (Ortiz et al, 1988; De Jong and Tai, 1991). Thirdly, germplasm enhancement can be more straight forward in potato because there are numerous valuable wild Solanum species which are diploid and can be more conveniently hybridized with diploid *S. tuberosum*. Such 2x-hybrids can be most valuable when they also produce 2n-gametes so that they can be readily used to produce tetraploids. Finally, sexual polyploids are more fertile than the colchicine doubled tetraploids. In view of these advantages of using 2n-gametes in potato breeding, it is important to examine the methods of selecting diploid potato clones that produce high frequencies of 2n-gametes.

Besides a high frequency, it is important to produce one or the other of the two genetically different types of 2n-gametes, viz., first division restitution (FDR) and second division restitution (SDR) gametes. Both of these types of gametes originate from meiotic division abnormalities. FDR gametes result from an equational division of the entire diploid compliment after pairing and crossing-over, giving rise to two genetically similar restitution nuclei. Such, an abnormal division can occur either during the first or the second meiotic division (Mok and Peloquin, 1975a; Ramanna, 1979; 1983). A notable feature of FDR gametes is that they mainly retain the parental gene combinations, including heterozygosity, intact and that they are genetically homogeneous. Some amount of segregation can occur in the case of FDR due to crossing-over. SDR gametes, on the contrary, originate through the restitution of the products of the reductional division. Theoretically, SDR gametes do not retain the parental gene combination. The 2n-gametes are expected to be largely homozygous and heterogeneous, i.e., their heterogenity results from crossing-over and random chromosome assortment. If the main aim is to obtain 4x- progeny that are highly vigorous, genetically uniform and retain the parental gene combinations intact, the FDR gametes are the most preferable one between these two types.

In view of the importance of 2n-gametes for breeding, it is necessary to combine many of the desirable agronomic traits with 2n-gamete formation in diploid potato. Prospects for the selection of such clones will be favourable if 2n-gamete formation in diploid potato were to be under simple genetic control. Several previous investigations (Jacobsen, 1976; Veilleux, 1982, and McHale, 1983) have shown that although 2n-gamete formation in potato is genetically controlled, their inheritance is by no means simple (Chapter 2). Because of the complicated nature of inheritance, a quantitative rather than a qualitative approach would be more appropriate for the selection of desirable diploid potato clones with high frequencies of 2n-gametes. The present investigation concerns the frequency of 2n-pollen producing plants and the frequencies of 2n-pollen formation in individual plants of different types of progenies of selected diploid parental clones, and their backcross derivatives. The implications of these results for true potato seed production and analytic breeding are discussed.

3.1. Material and methods

Four different types of diploid populations were investigated for estimating the frequencies of plants that produced varying levels of 2n-pollen. These populations were produced by intercrossing the following types of diploid clones: 1) Three clones of *Solanum phureja*, IvP35, IvP48 (Hermsen and Verdenius, 1973) and IvP101 (Hutten et al., 1994) all of which produced either low frequencies or no 2n-pollen. These progenies are indicated together as "low x low" (Table 3.1). 2) The three IvP clones of item 1 were crossed with three selected clones, USW5293-3 (A), USW5337-3 (C) and 77-2102-37 (E) which produced fairly high frequencies (5% or more) of 2n-pollen. These resulted in nine combinations and are indicated as "low x high". 3) The three selected clones, A, C, E, including two additional clones, USW5295-7 (B) and USW7589-2 (D) (Fig.3.1., Jongedijk and Ramanna, 1988) were crossed in an incomplete diallel of "high x high" combination and consisted of 13 combinations. These are indicated as "high x high" progeny. 4) Some of the selected hybrids between the clones mentioned in item 3 were backcrossed to one of the parents. These are indicated as "high x high-backcross".

All crosses were made at the Department of Plant Breeding, Agricultural University of Wageningen. The progeny populations were grown at the Research Station for Highland Agriculture in Zhangbei, Hebei province of China. The populations were grown during 1993 as seedlings and in 1994 from tubers for the estimation of 2n-pollen frequencies.

Pollen grains from fully mature flowers were mounted in a drop of 2% acetocarmine solution in order to estimate the frequencies of 2n-pollen. All those grains that exceeded a diameter of 23 µm and had 4 or more germpores were considered 2n-pollen and smaller ones as n-pollen. Generally, about 500 pollen grains were counted for estimating the frequency of 2n-pollen in all those progeny that flowered.

Cytological modes of origin of 2n-pollen was monitored in the parental clones and the progenies according to Ramanna (1979). Briefly, this technique was as follows: young anthers were fixed in a freshly prepared 3:1 solution of ethanol: propionic acid saturated with ferric acetate. After allowing anthers in the fixative for a week or longer, parts of anthers were squashed in a drop of 2% acetocarmine on a microscopic slide. Metaphase II stages were examined for spindle abnormalities. The presence of fused spindles was considered as an indication for FDR mode of origin of 2n-pollen.

The percentage of plants producing 2n-pollen were calculated and 2n-pollen distribution were charted along with X axis of intervals as <1%, 1.1-5%, 5.1-10%, 10.1-15%, 15.1-20%, 20.1-25%, 25.1-30%, and >30%. The general trends in the different populations and combinations were compared.

3.2. Results

The four types of crosses were made on the basis of previously available information regarding the frequencies of 2n-pollen formation in all diploid parental clones used in this investigation. The IvP clones represented the class of parents that produced low levels or no 2n-pollen. The A, B, C, D and E clones together with some of the hybrid progenies (EC, EB, CD, ED, CB) produced 5 % or more 2n-pollen. The cytological examination indicated that meiosis was completely normal in IvP clones except for a low frequency of fused spindle formation during metaphase II stages. In all other parental clones varying frequencies of 2n-pollen formation were observed (data not presented) and in all cases fused spindle formation was found to be the main cause of 2n-pollen formation. Thus, in these parental clones 2n-pollen originated predominantly through FDR suggesting that the same alleles leading to this mechanism are involved.

Data on 2n-pollen production in four populations collected during two seasons were nearly concurrent. Therefore, the average values of two seasons were used for indicating the frequency distribution of plants with varying levels of 2n-pollen formation (Table 3.2). There were considerable differences among the four types of populations with regard to the frequencies of 2n-pollen producing plants. A notable feature was that in all combinations the range of 2n-pollen frequencies varied, the maximum being 85.7% (Table 3.2). Even in low x low combinations there were plants in which a maximum of 30.4% of 2n-pollen was observed. However, the frequencies of plants with low percentages, or no 2n-pollen predominated (57.5%) in low x low population, which was represented by the progeny of IvP clones (Table 3.2). In the other three types of combinations, the frequency of plants with higher percentages of 2n-pollen varied considerably in all frequency distribution intervals (see standard deviations in Table 3.2). However, there was a steady increase in the mean frequencies of 2n-pollen in the successive combinations of low x high; high x high and high x high-backcross (Table 3.2). There was a clear response to repeated cycles of selection for increased 2n-pollen frequencies Thus the progeny from crosses between IvP clones and A, C and E clones constituted the combinations of low x high. In this population, a much lower percentage (27.4%) of plants produced less than 1% 2n-pollen, i.e., a much higher proportion of plants produced 2n-pollen than the previous population. The third type of population, high x high combinations, was derived from intercrossing of clones A, B, C, D and E all of which were selected for the production of 2n-pollen, albeit with variable frequencies. In the progenies of crosses of these clones, the percentage of plants that produced less than 1 % 2n-pollen was in a lower proportion as compared to the low x high type of population. Conversely, the percentage of plants that produced more than 5% 2n-pollen was higher in comparison to the two previous populations. Finally, the high x high-backcross populations were derived from crossing some of the selected clones from the high x high combinations with one of the parental clones (e.g., C x EC; EC x C; ED x E; EC x E; EC x EB). With a few exceptions, they were backcrosses. In the progeny of high x high-backcross population, only 15.7 % of plants produced lower than 1% of 2n-pollen and the proportion of plants that produced 5 % or more 2n-pollen was much larger than in any of the other populations (Table 3.2)

In order to assess the effectiveness of using selected diploid parental clones for increasing the frequencies of 2n-pollen in the progeny, a comparison was made of the percentage of plants that produced more than 25 % 2n-pollen in different populations (Table 3.2). There was a fourfold increase of the percentage of 2n-pollen in the type of high x high-backcross population as compared to low x low. So also, there was a notable increase of the mean of the maximum percentage of 2n-pollen frequency for the population as well as the population mean percentages progressively. Here standard deviation for maximum frequency of 2n-pollen were quite high (9.22-19.30). If the individual population was assessed, there was a clear difference of maximum frequency of 2n-pollen formation among combinations as indicated by the high standard deviation (Table 3.2).

3.3. Discussion

The diploid clones used in this investigation are of complex hybrid origin with the exception of IvP clones. Despite this, they all have one common feature: in their pedigrees, the primitive cultivated diploid species *S. phureja* is involved (Fig.1). The three diploid clones, IvP35, IvP48 and IvP101, produced low frequencies of 2n-pollen. This is not unexpected because these clones have been indirectly selected for a lower frequency of 2n-pollen production (Hermsen and Verdenius, 1973) in view of using them as pollinators for induction of parthenogenetic dihaploids. This implies that there might have been a loss of alleles determining the frequency of 2n-pollen formation in these clones but that the potential via the FDR mechanism still exists. If this is the case, the high frequency of plants in low x low population (Table 3.2) which produced either low frequencies or no 2n-pollen is explainable on the assumption that these clones lack some of the alleles necessary for 2n-pollen formation. It should also be pointed out that in some circumstances one of the clones, IvP35, has produced fused spindles and 2n-pollen (Ramanna, 1979). From the pattern of segregation for plants that produce less than 1% or none and those that produce 2n-pollen it is evident that this trait is not under monogenic control, contrary to that reported earlier (Mok and Peloquin, 1975a and 1975b). It is probable that 2n-pollen formation in diploids is controlled by a major gene whose action is greatly modified by several minor genes. If this is the case, a combination of several genes is required for disturbing the process of meiosis and give rise to 2n-pollen.

Since *S. phureja* is involved in the pedigrees, the progeny populations from low x high (IvP clones x A, E and E) combinations can be regarded as backcrosses. Because the A, C and E clones were selected for 2n-pollen production and when these are backcrossed to IvP clones, the progenies are expected to show an increased average percentage of 2n-pollen if there was any effect of recurrent parents. Indeed, a clear increase in the frequencies of plants with 2n-pollen production and of plants with relatively high 2n-pollen frequencies are evident in the set of population of low x high combinations. The same trend is reflected in high x high-backcross populations which are derived from backcrossing selected parents such as EC, EB, CD, ED and CB with C and E clones. In these progenies, the increase of the frequencies of 2n-pollen is much more pronounced because it amounts to fourfold increase as compared to the low x low combinations (Table 3.2). Thus, depending upon the performance of the progeny populations, it should be possible to select the most favourable parents that can give rise to promising progenies. These results are in agreement with those reported for Red Clover (Parrot and Smith, 1986), alfalfa (Tavoletti et al., 1991) and potato (Jacobsen, 1976; Ortiz and Peloquin, 1992; Barone et al., 1993), where repeated cycles of selection has lead to the increase of the frequency of 2n-pollen.

Although the genetic control of 2n-pollen formation is complicated, the positive response observed in the case of repeated cycles of selection suggests that it is possible to select genotypes with higher frequencies of 2n-pollen. It is likely that some major genes that are highly influenced by several minor genes might determine the frequencies of 2n-pollen. If this is the case, as a result of repeated cycles of selection, the populations are enriched for the minor genes so that the frequencies of 2n-pollen are increased. However, because of the quantitative inheritance as well as the complicated expressivity of 2n-pollen formation the selection for this character is difficult. Once the appropriate genotypes are selected, as has been done in the case of diploid genotypes of this investigation, it is possible to improve the populations more predictably.

From the point of view of potato breeding, all four types of diploid populations might be interesting because of the following reasons: a) In all cases, *S. phureja* is involved as a parent with the dihaploids of the cultivated potato. Thus, agronomically, these populations are not far fetched from the cultivated potato. This means, they can be directly used in breeding. b) Most of the parental clones have been analysed cytologically (Ramanna, 1979; Jongedijk and Ramanna, 1988; Hutten, 1994) and the mode of origin of 2n-pollen is predominantly FDR. This could indicate that for this particular trait the same set of multi-alleles is involved. c) Some of the tuber characteristics from *S. phureja* that are present in these populations can be valuable when incorporated into the cultivars.

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Table 3.1 List of parents used in the four types of populations.

Population	Parents involved
Low x Low	IvP35, IvP48, IvP101; Lines from *S. phureja*
Low x High	IvP35, IvP48, IvP101; A, C, E clones.
High x high	A, B, C, D, and E clones.
High x high-backcross	EC, EB, CD, ED, CB; C, E

EC, EB, CD, ED and CB came from the cross of E, C, B and D clones.
Low = <1%, high = >5%.

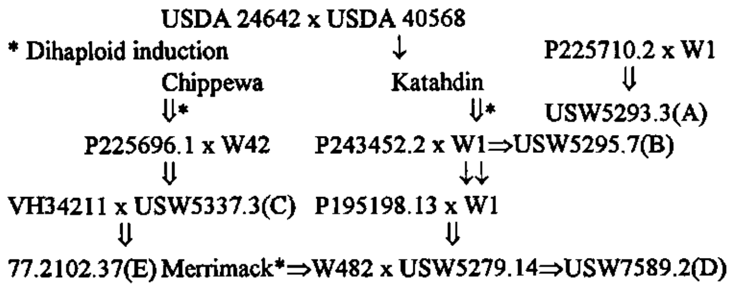

Figure. 3.1. The genetic background of the diploid potato clones USW5293.3(A), USW5295.7(B), USW5337.3(C), USW7589.2(D), and 77.2102.37(E); here P = *S. phureja*; V = *S. vernei* and H = *S. tuberosum* dihaploid (modified from Jongedijk and Ramanna, 1988).

Table 3.2 Effectiveness of repeated cylces of phenotypic selection for 2n-pollen formation compared in four different types of populations.

Type of populations	Number of combinations	Frequency distribution of plants producing 2n-pollen <1%	1.1--5%	>5%	>25%	Xmaxi.(%)	Xmean (%)	Range* highest frequency (%)	No.plants analysed
Low x Low	3	57.5±21.21	17.1±15.34	25.4	4.5	18.8± 9.22	2.37±0.99	8.6-30.4	132
Low x High	9	27.4 ±7.41	25.0 ±4.76	47.6	7.0	41.6± 12.74	6.66±1.18	27.3-75.0	1539
High x High	13	21.6±12.25	22.9 ±11.33	55.5	11.7	46.6± 19.30	8.68±2.69	13.8-85.7	1692
High x High-backcross	8	15.7±8.13	20.5 ±11.98	63.8	17.5	42.2± 16.31	10.70±4.67	18.0-68.0	608

* range for highest frequency of each combination in the population.

"Low x Low" population: all parents produced very low or no frequencies of 2n-pollen;

"Low x High" population: the males had ability to produce 2n-pollen; females not;

"High x High" population: selected males and females could produce certain 2n-pollen;

"High x High backcross" population: the parents were selected from High x high progeny population and crossed with their parent, respectively.

Chapter 4

Effective transfer of bacterial wilt (*Pseudomonas solanacearum E. F. Smith*) resistance to sexual offspring in 4x-2x crosses by 2n-pollen

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(Submitted)

Summary

Twenty six potato clones, including 18 diploid and eight tetraploid ones, were screened for resistance to bacterial wilt (BW) caused by *Pseudomonas solanacearum*. A range of phenotypes varying from highly resistant to completely susceptible was found. Among the diploid resistant clones, 2n-pollen producing genotypes were selected. When the 2n-pollen producing BW resistant diploid clones were used as male parents in 4x-2x crosses (unilateral sexual polyploidization), the disease incidence and disease index in the progenies were lower than those in the progenies of 2x-2x crosses. A comparison between 4x-2x and 4x-4x crosses for transfer of BW resistance into the offspring showed that 4x-2x crosses were more effective. Besides transferring BW resistance, 2n-gametes originating through mechanisms leading to FDR can also be of advantage for maximizing heterozygosity.

Key words: Bacterial wilt resistance, unreduced gametes, unilateral sexual polyploidization, diploid potato; *Solanum tuberosum* and *Pseudomonas solanacearum*.

Introduction

Bacterial wilt of plants, caused by *Pseudomonas solanacearum E. F. Smith*, is a wide spread disease affecting numerous plants from nearly 44 different plant families. Although this pathogen occurs all over the world (45^0 S to 59^0 N), it is mainly epidemic in hot rainy tropical and subtropical, but partly also in temperate regions. The first report on bacterial wilt in China appeared in early 1940s (He, 1994). Instances of affecting crops such as tobacco, tomato, eggplant, chilli pepper, peanut, potato and sweet potato were evident in the eight provinces south of the Yangtze river (He, 1994). By 1980's, however, it was established that more than 30 plant species in 17 provinces, extending to the North of the Great Wall, Inner Mongolia, and the NingXia autonomous region, were affected by bacterial wilt (He, 1994, Fong, 1993). Because this disease is systemic, the plants are killed and the damage generally amounts to 10-30%, and in most serious cases it can be as high as 80%. This disease is prevalent in the South, North and Southwest of China where 50% of the national potato acreage is situated and thus vulnerable to this disease.

There are different races of *P. solanacearum* such as Race 1, 2, 3, and 4 that infect different hosts. Race 1 infects most crops, i.e., a wide range of hosts, Race 2 infects banana and *Heliconia,* Race 3 infects potato, and incidentally tomato and eggplant (Buddenhagen et al., 1962). Race 4 was reported to infect ginger in Philippines (Zehr, 1969). Among these, Race 3 is the predominant pathogen that causes the bacterial wilt disease of potato from Guandong to Bashang of Hebei province of China where it covers the tropical until temperate regions.

Although the pathology, epidemology and control of bacterial wilt were investigated almost 100 years ago (Smith, 1896), very little was known about the sources and genetics of resistance of this disease in potato until recently (Thurston and Lugano, 1968; Gomez et al., 1983; He, 1983; Schmiediche, 1988; Tung, 1992). Some of the diploid (2n=2x=24) primitive cultivated species such as *Solanum stenotomum, S. phureja* and diploid wild species *S. sparsipilum, S. chacoense, S. microdontum, S. jamesii* and the tetraploid (2n=4x=48) wild species *S. stenotomum* were reported to possess resistance against *P. solanacearum*, Race 3 (Martin et al., 1980; Thurston Lugano, 1968). The mode of inheritance of resistance in the available material is not known. However, some of the investigations in the past indicated that the inheritance is quantitative (Rowe and Sequeira, 1970; Schmiediche, 1988; Tung et al., 1990; He, 1983).

Because of the quantitative nature of inheritance it can be difficult to breed for resistance against bacterial wilt in cultivated potato. However, another approach could be to use the numerically unreduced (2n-) gametes that are more suitable for transferring quantitative traits. In this context, the so-called first division restitution(FDR) gametes are especially relevant because they can retain the parental gene combinations nearly intact and transmit to the sexual polyploid progeny in 4x-2x crosses (Mendiburu and Peloquin, 1977). In order to exploit the possibilities of transferring resistance against *P. solanacearum* into the potato material suitable for Chinese conditions clones that are resistant and also produce 2n-pollen were investigated.

4.2. Material and methods

4.2.1. Material

Plant material consisted of both diploid (2n=2x=24) and tetraploid (2n=4x=48) genotypes. Among diploids, there were 18 clones derived from diverse interspecific origin. These included : a). The five well-known clones USW5293-3 (A); USW5295-7 (B); USW5337-3 (C); USW7589-2 (D) and 772102-37 (E) which were developed at the University of Wisconsin (USA), the Max Planck Institute, Cologne (Germany) and at the Department of Plant Breeding (IvP), Wageningen Agricultural University (the Netherlands) (Jongedijk and Ramanna, 1988). In all these cases *S. phureja* was involved as one of the parents. In the pedigree of clone E, *S. vernei* was also involved. b). Five diploid hybrid progenies of some of the clones mentioned in the item a. These were CD1045, CD1047, CE13, CE1063 and ED1022. c).Q87350-3 (SH82-59-223 x VRN15451-14 which has the genetic background of *S. vernei*) and Q87174-7 (MCD18302-33 x SH82-59-223 which has the genetic background of *S. microdontum*). d). Two clones of the diploid species *S. phureja*, IvP35 and IvP48. All materials of items b, c and d were selected at the Department of Plant Breeding (IvP), WAU, Wageningen (the Netherlands) and. e). Four clones, D-2-1, D-5-1, D-6-1 and D-7-1, all of which were developed at the Institute of Vegetables and Flowers (IVF), CAAS, Beijing (China) and included the three diploid species, *S. phureja, S. chacoense* and *S. goniocalyx* in their pedigrees.

There were eight tetraploid genotypes of which Q9201-12 and B927017-10 were sexual polyploid derivatives of the clones CD1045 and ED1045 from IvP, Wageningen (the Netherlands). AVRDC1287 (as resistant control), MS1C.2 and MS42.3 were received from CIP, Lima, Peru. Zhongshu No2 (as susceptible control) and W2 were made available from IVF, Beijing. Asterix is a Dutch potato cultivar.

A number of 4x-2x and 2x-2x crosses were made in the greenhouse at IvP, Wageningen and Bashang, Hebei. Eleven 4x-2x hybrid combinations and thirteen

2x-2x combinations were selected for seedling transplants and their tubers were harvested and used for the evaluation of resistance trials.

4.2.2. Testing for resistance against bacterial wilt (BW)

The tubers of all progeny plants and at least five tubers for each genotype were sown in pots consisting of sterile substrate made of peat and Vermiculite. Once the plants attained a height of about 10 cm with 7-8 leaves, the entire root system was treated with a slurry of *Pseudomonas solanacearum* at a concentration of 10^5 Colony Forming Unit(CFU)/ml (30 ml for each plant) for five minutes. Treated plants were transplanted into new pots with new substrate and the leftover slurry was filled into pots. All inoculated plants were moved into a greenhouse, the temperature of which was maintained at 28 ± 1^0 C and the light intensity was regulated to be 2000 lux during the day. The relative humidity was maintained at 85%. The infected plants and their severity were recorded during three intervals of 5, 8 and 12 days after inoculation (Fong, 1993).

The infection grade was classified as follows:
0= no symptom
1= wilting with yellowish lower one or two leaves
2= wilting of 3 or 4 leaves
3= wilting of 50% or more leaves
4= wilting of all leaves
5= plant dying with all leaves wilting.

Disease index (%) = $\sum$[No. plants x infection grade]/ 5 x total plants.

Relative resistance (RR) was calculated based on following formula:

Relative resistance (RR) =

$$\frac{\text{Disease index of susceptible check - index of testing material}}{\text{Disease index of susceptible control}} \times 100\%$$

RR value:		
	> 80%	high resistance (HR)
	80-60	resistance (R)
	60-40	medium resistance (MR)
	40-20	medium susceptible (MS)
	<20%	susceptible (S)

4.3. Results

As a first step, eighteen diploid and eight tetraploid clones were screened for BW resistance and classified according to the incidence and disease index (Table 4.1). Among the 18 diploid clones tested in this investigation, more than half of them were found to possess resistance. One of them was highly resistant (ED1022), two were resistant (CD1045, Q87174-7), six were medium resistant and five were susceptible. A notable feature was that the highly resistant clone ED1022 which produced FDR 2n-pollen was derived from a cross between clones that were medium resistant (E) and susceptible (D), respectively. The susceptible clone CE13 was derived from a cross between two medium resistant clones C and E. Thus, it was evident from these observations that segregation of different detectable phenotypes was possible using these clones. Some of the resistant clones produced sufficient 2n-pollen so that they could be successfully used as male parents in 4x-2x crosses (Table 4.2). In the resulting tetraploid progenies the notable differences were found with respect to BW resistance.

As in the diploids, four phenotypic classes ranging from highly resistant to susceptible were found among the tetraploid parental clones tested (Table 4.1).

In order to monitor the transfer of BW resistance to the tetraploid progenies different types of crosses were made and progenies were tested. These were in 4x-2x crosses: i) resistant x resistant (4x R-2x R); ii) susceptible x resistant (4x S-2x R); iii) resistant x susceptible (4x R-2x S) and iv) susceptible x susceptible (4x S -2x S) combinations, and finally in 4x-4x crosses with three different combinations, viz., resistant x susceptible, susceptible x resistant, susceptible x susceptible. The disease rate and disease index for the assorted groups of different types of crosses are given in Table 4.2. It was evident that there were clear differences among different groups of progenies with respect to incidence of disease expressed in percentage of infected plants (%) and the disease index observed in three different intervals at five, eight and twelve days after inoculation. The tetraploid progenies from 4x-2x crosses with R x R combinations gave the lowest disease infection and also a lower index at five days after inoculation but it was not much different from that in S x R combinations. That trend occurred until 8 and 12 days after inoculation. Furthermore, both of them were much less susceptible to BW than that of R x S combinations with a susceptible diploid male (2n-pollen producer). However, 4x-2x families with diploid susceptible male parents also had a lower disease rate and index than that of the susceptible 4x-4x check. These observations indicated that FDR 2n-pollen can transmit bacterial wilt resistance from diploids to tetraploid progenies by 4x-2x crosses, even if female tetraploids possessed susceptibility. However, the resistance transmitted by tetraploids was very limited (see the case of R x S type). In all, comparatively lower rates of infection and lower disease indexes were observed in progenies of both 4x R-2x R and 4x S-2x R combinations. However, the incidence and disease index were higher in other 4x-2x combinations including R x S and S x S. The highest incidence and index of the disease were observed in all counterparts of 4x-4x combinations. The diploid clone CD1045 lead to progenies with a high level of resistance reflecting the effective transmission of this quantitative trait in different cross combinations. There were clear indications that when 2n-pollen grains from the resistant genotypes were used in 4x-2x combinations, the resulting 4x progenies frequently had a lower disease incidence as well as a lower disease index (Table 4.2).

Beside 4x-2x crosses (unilateral sexual polyploidization), it was possible to generate and improve the resistance at diploid level by intermating diploid genotypes. From such crosses, seven different diploid progenies were tested for BW resistance (Table 4.2). The incidence of disease and the disease index were comparatively higher in all groups of progenies (R x R, R x S, S x R, and S x S) as compared to those in 4x-2x progenies (R x R, S x R), indicating that different resistance factors were involved in both parents. But it should be emphasized that diploid progenies from R x R, S x R and R x S 2x-2x crosses showed relatively higher tolerance after 5-8 days of inoculation than with those of S x S crosses

4.4. Discussion

From a systematic screening of 26 potato clones of diverse origin for BW resistance it was evident that there was a range of phenotypes showing high resistance to complete susceptibility (Table 4.1). These results, together with the data on segregation of BW resistance in the diploid and 4x progenies of 2x-2x and 4x-4x crosses, respectively (Table 4.2) clearly indicate that the inheritance of BW resistance in potato is complex. This observation agrees with the previously published results (Rowe and Sequeira, 1970; Schmiediche, 1988; Tung et al., 1990; Pham Xuam Tung, 1992; He L.Y. et al., 1994), which complicates the introduction of BW resistance into the cultivated potato through traditional breeding methods.

One potential approach to overcome this problem is to use 2n-gametes which have been shown to be successful for transferring other quantitatively inherited diseases in potato. For example, resistances against root-knot nematodes (Iwanaga et al., 1989), potato cyst nematodes (De Maine et al., 1986), early blight (Herriott and Haynes, 1990) and bacterial wilt (Watanabe et al., 1992), which are quantitatively inherited, have been shown to be amenable for transfer through 2n-gametes.

In two instances, where the 2n-pollen producing male parents were resistant, the 4x progenies had a relatively lower incidence of disease as well as a lower disease index (Table 4.2). On the other hand, in two groups of progenies which resulted from 4x-2x crosses involving susceptible pollen parents, the incidence of disease and disease index were clearly higher. This means that in the two cases (R x R and S x R) where the pollen parent was resistant the BW resistance is frequently transferred effectively through 2n-pollen. As a comparison, the progenies of 4x-4x crosses (Table 4.2) had a much higher disease incidence as well as a disease index regardless of the types of parents involved (R x S, S x R and S x S).

Potato germplasm enhancement by using wild or related species can be achieved at the diploid level quicker than by the conventional breeding approach. In the improved materials the desirable traits from wild or exotic genetic stocks at the diploid level would be transferred to tetraploid potatoes by adopting 2n-gametes. Both 2n-pollen and 2n-eggs can be used successfully for polyploidization via 4x-2x and 2x-2x crosses. The advantages of 2n-pollen over 2n-eggs for transferring traits to the polyploidized offspring have been discussed by Iwanaga et al., (1989). 2n-pollen produced by a modified meiosis which is genetically equivalent to First Division Restitution (FDR), can preserve and transmit the parental genetic constitution to the offspring at a high degree (Ramanna, 1992; Jacobsen et al., 1991; Peloquin et al., 1989; Qu et al., 1993). Even with quantitatively inherited traits, such as resistance to root-knot nematodes (Iwanaga et al., 1989) and early blight (Herriott et al 1990), FDR 2n-gametes have a big potential for transferring major components of genetic factors from diploid parents to their tetraploid progeny via 4x-2x crosses. This presentation is an additional example to show this important phenomenon. The challenge will be to combine resistances, 2n-gametes and acceptable agronomic traits in one and the same diploid parental genotype which can also be the male parent for improved TPS varieties.

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Table 4.1. Performance of diploid and tetraploid potato parental clones for bacterial wilt resistance tested at 5, 8 and 12 days after inoculations.

Parent	Source	Plants tested	5DIN		8DIN		12DIN		Status
			%	Ind	%	Ind	%	Ind	
2x lines									
A	USW	10	30	12	30	22	50	50	MR
B	USW	6	100	33	100	47	100	47	MS
C	USW	7	14	10	29	34	57	46	MR
D	USW	8	50	20	50	40	100	90	S
E	IvP	8	0	0	50	35	63	45	MR
CD1045	IvP	11	27	27	33	31	56	38	R
CD1047	IvP	11	50	22	60	44	90	78	MS
CE1063	IvP	10	20	4	50	16	50	42	MR
ED1022	IvP	5	0	0	0	0	0	0	HR
CE13	IvP	9	78	44	100	80	100	83	S
Q87350-3	IvP	10	50	20	100	80	100	100	S
Q87174-7	IvP	8	0	0	25	15	50	30	R
IvP35	IvP	6	100	100	100	100	100	100	S
IvP48	IvP	6	100	100	100	100	100	100	S
D-2-1	IVF	13	54	15	62	34	62	46	MR
D-5-1	IVF	9	44	16	89	42	89	53	MR
D-6-1	IVF	9	56	46	67	58	89	71	MS
D-7-1	IVF	9	100	100	100	100	100	100	S
4x lines									
Q9201-12	IVF	5	0	0	0	0	20	25	R
B927017-10	IvP	5	40	10	80	50	100	75	MS
[1]AVRDC1287	CIP	8	0	0	25	20	25	25	R
MS1C.2	CIP	10	0	0	30	15	30	20	HR
MS42.3	CIP	10	0	0	0	0	20	15	HR
[2]Zhongshu No2	IVF	6	50	20	100	80	100	100	S
Asterix	IvP	6	33	7	100	100	100	100	S
W2	IVF	10	50	18	100	70	100	90	S

% = disease incidence rate; Ind = disease index; DIN = days after inoculation.

1. AVRDC1287 as resistant control,

2. Zhongshu No.2 as susceptible control.

R=resistant, S=susceptible, MS= medium resistant, MR=medium resistant, HR=highly resistant.

Table 4. 2 Reaction of 4x potato progenies of 4x-2x , 4x-4x crosses and of 2x potato progenies of 2x-2x crosses in assorted groups to bacterial wilt at 5, 8 and 12 days after inoculation.

Cross	No.plants	5DIN		8DIN		12DIN	
	tested	%	Ind	%	Ind	%	Ind
*4x-2x with R x R**							
AVRDC1287 x CD1045	40	0	0	31	7	82	32
MS1C-2 x CD1045	27	11	3	70	32	100	69
MS42.3 x CD1045	32	4	1	53	16	78	34
AVRDC1287 x ED1022	20	0	0	30	5	100	33
Sub mean		4	1	46	15	90	42
4x-2x with S x R							
W2 x D-2-1	12	17	10	42	20	75	53
B927017-10 x CD1045	20	0	0	50	20	50	30
Sub mean		9	5	46	20	63	42
4x-2x with R x S							
AVRDC1287 x CE13	16	24	10	81	43	91	43
AVRDC1287 x CD1047	26	15	5	35	9	100	43
MS1C-2 x CD1047	33	39	9	88	58	95	77
Sub mean		26	8	68	37	95	54
4x-2x with S x S							
Q87350-3 x B927017-10	15	40	8	60	12	82	32
Zhongshu No.2 x D-7-1	12	25	10	92	57	100	92
Sub mean		33	9	76	35	91	62
4x-4x							
AVRDC1287 x Zhongshu No.2(R x S)	20	40	10	75	30	100	85
Asterix x Q9201-12 (S x R)	34	22	10	44	33	100	76
Asterix x Zhongshu No.2 (S x S)	16	50	20	75	30	100	95
Sub mean		34	13	65	31	100	85
2x-2x with R x R							
E x A (R x R)	12	28	18	83	57	91	81
2x-2x with R x S							
D-2-1 x D-6-1 (R x S)	53	43	28	95	66	100	91
Q87174-7 x IvP48 (R x S)	28	6	1	68	24	87	43
Sub mean		25	15	82	45	94	67
2x-2x with S x R							
D x C (S x R)	13	21	12	89	48	100	84
D x E (S x R)	9	17	6	49	21	90	57
Sub mean		19	9	69	35	95	71
2x-2x with S x S							
D x B (S x S)	11	41	28	91	66	97	85
D-5-1 x D-6-1 (S x S)	43	45	18	80	48	88	84
Sub mean		43	23	86	57	93	85

% = disease incidence rate; Ind = disease index; DIN = days after inoculation.

1. AVRDC1287 as resistant control,

2. Zhongshu No.2 as susceptible control.

* R = resistant, S = susceptible.

Chapter 5

Exploitation of the progeny from 4x-2x crosses of potato for analytic breeding and true potato seed production

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Summary

In order to explore the possibilities for true potato seed (TPS) production as well as analytic breeding, three different types of 4x populations were evaluated. These populations consisted of progenies from: 1) 4x-2x, 2) 4x-4x crosses and 3) 4x-selfings. All three types of progenies were grown as seedling and clonal generation during 1993 and 1994, respectively. In both generations, a total of 15 traits, including agronomic characters such as the number and yield of tubers per plant, late blight resistance, maturity and specific gravity of tubers, were evaluated. Significant variation was observed for various traits evaluated. Although there was uniformity for certain traits in some of the combinations, none had uniformity for several characters at the same time. General and specific combining abilities (GCA; SCA) were calculated for four agronomic traits in the progenies of 4x-2x crosses. Some of the female parents such as Escort, Katahdin and Premiere showed higher GCA values for tuber yield but were not consistent with the cases of specific gravity. In general, Premiere, Desiree and Escort were good female parents for TPS production and FB155, FB122, CD1045 and CB122 had the potential for being diploid male parents. An evaluation of agronomic characters indicated that some of the combinations of 4x-2x crosses offer great potential for TPS utilization as a variety. These observations might be helpful to improve the populations which are needed in the case of TPS production. SCA analyses for agronomic traits such as tuber yield and specific gravity were relevant for selection of hybrid combinations as well as analytic breeding.

Keywords: *Solanum tuberosum*, progeny evaluation, 4x-2x crosses, parental value, unreduced gametes, diploid potato, true potato seed, analytic breeding and combining ability.

Introduction

The cultivated potato, *Solanum tuberosum*, traditionally is a vegetatively propagated autotetraploid (2n=4x=48). With rare exceptions, this crop is propagated almost exclusively through planting of the tubers. For the purpose of breeding, however, developing of plants from true botanical seed is essential. Apart from this, attempts have also been made in the past to cultivate potato through true potato seed (TPS) rather than planting of tubers but with limited success. In recent years, however, there has been growing interest to produce potato cultivars that can be propagated through botanical seed. In fact, remarkable success has been achieved in China and India in growing potato through true botanical seed (Li, et al., 1978, Sawyer, 1984, Song et al, 1987). Since then, production of potato from seedling transplants or tuberlets has been realized and extended into other developing countries (Pallais, 1994).

There are both advantages and disadvantages in using either tubers or true potato seed as planting material. The advantages of using tubers as planting material are that 1) because of vegetative propagation, the crop will consist of clonal populations of plants so that they will be highly uniform; 2) the plants will be highly vigorous; 3) high crop yields can be obtained in a relatively short period. The disadvantages are that the tubers can be infected easily with viral, bacterial and fungal organisms and the planting material can "degenerate". This means that it is essential to use only healthy planting material each time, which can be expensive. Moreover, great quantities of tubers used for propagation could otherwise be used as food. The advantages of growing potato with TPS are that they are highly inexpensive; disease transmission, especially viruses, is minimal; the growers can produce their own planting material without involving advanced technologies. The disadvantages of growing potato through botanical seed are that the crop can be highly heterogeneous, that require more efforts and time to grow a crop from seeds and seedling stages.

Depending upon whether a potato cultivar is to be propagated through tubers or botanical seed, the approaches required for breeding are different. In the case of tuber propagated cultivars, traditionally, 4x-4x crosses were made and from a large population of the resulting progeny few desirable genotypes were selected, and the most promising ones among these were clonally propagated as cultivars. One of the drawbacks of this method was that potato is a polysomic polyploid and its pattern of inheritance of characters is highly complicated as a result of which it was not easy to select the desirable combinations of the phenotypes. To overcome this bottle neck, Chase (1963) suggested a method called "analytic breeding" for potato. According to this approach, the tetraploids are reduced to diploid condition (2n=2x=24); breeding is conducted at the diploid level and, by using the improved diploids, tetraploids are produced through the use of 2n-gametes. As an extension of this scheme, the so-called unilateral (4x-2x) and bilateral (2x-2x) sexual polyploidization methods have been proposed (Mendiburu and Peloquin, 1974; Kidane-Mariam and Peloquin, 1974). As a result of this development, breeding of potato at the diploid level has become attractive for the breeders (Hutten, 1994).

Unlike analytic breeding in which only a few genotypes are selected for clonal multiplication, breeding for true potato seed (TPS) cultivars requires the improvement of the whole population. To this end, several approaches and requirements have been considered in the past (Hermsen et al., 1985; Tai et al., 1992; Werner and Peloquin, 1992; Clulow et al., 1995; Almekinder, 1995). In this investigation, the prospects for both analytic breeding as well as TPS cultivar production by using different types of crosses, with emphasis on 4x-2x crosses, are considered.

5.2. Material and methods

Sixteen diploid and fourteen tetraploid genotypes were used in this investigation. The diploids were complex hybrids derived from crossing diploid *S. tuberosum* with some of the diploid primitive cultivated species, viz., *S. phureja*, *S. goniocalyx* and diploid wild species *S. chacoense* and *S. vernei*. The genotypes included USW5293-3 (A), USW5295-7 (B), USW5337-3 (C), USW7589-2 (D) and 772102-37 (E); and some of the diploid F1 hybrids of the above mentioned genotypes: CD1045, CE10, CE1063, EC394, ED1022, ED1045, FB155, BE62, CB32, CB122 and Q9201-59. All these genotypes were previously selected for the formation of 2n-pollen (Ramanna, 1983, Jacobsen, 1978, Mok and Peloquin, 1974, Hutten, 1994) and the cytological modes of origin of 2n-pollen was known (Jongedijk and Ramanna, 1988). Two clones among these, CE10 and BE62, were known to produce exclusively FDR pollen grains because of their homozygosity for desynapsis (ds/ds) (Ramanna, 1983).

The tetraploid genotypes used here were all cultivars (Asterix, Chippewa, Desiree, Eba, Escort, Estima, Gineke, Isola, Katahdin, Merrimack, Premiere) with one exception, R16B-19 (Escort x BE1073). The cultivars included both early and late maturing types.

Using the above mentioned diploid and tetraploid genotypes, three types of crosses were made at the Department of Plant Breeding, WAU, Wageningen and at the Institute of Vegetables and Flowers in Beijing. i) 4x-2x crosses in which 14 tetraploids were used as female parents and crossed with 16 diploid genotypes that produced 2n-pollen grains. ii) 4x-4x crosses in which five cultivars and three 4x clones derived from the bilateral sexual polyploidization of well-known diploid clones. iii) selfing of four tetraploid cultivars, Asterix, Kondor, Katahdin and Desiree.

Since all the plant materials described above were selected and tested in Europe, some locally tested diploid and tetraploid genotypes were used as controls for comparison. D-2-1, D-6-1 and D-7-1 were all diploids producing 2n-pollen and W2, Mira and Spunta that were tetraploids.

In order to grow plants from true seed, they were germinated as described in chapter 2. About six weeks old seedlings were transplanted into the fields. 34 crosses of 4x-2x and 5 combinations from 4x-4x and 4 selfings seeds were sown. In each case, 100-200 seedlings per combination were planted into the fields in 1993 and at maturity tubers of each plant were harvested. From each genotype one tuber was collected. These tubers were pooled together and constituted the "tuber family". In 1994, 34 tuber families plus 5 check combinations of 4x-2x and 4 combinations of 4x-4x along with 4 selfings were planted in single-row plots with 27 cm spacing between plants in rows of 67cm spacing between adjacent rows. For statistical analyses 30 plants were selected randomly from each experiment unit with Randomized Complete Block Design of three replicate blocks. The field trials were conducted at Bashang (41^0 N, 1400m ASL, single cropping region).

Methods of evaluation of agronomic traits

Seedling populations as well as tuber families from 35 combinations of 4x-2x crosses were evaluated for the following 12 characteristics during 1993 and 1994, respectively.

1. germination rate - recorded at five days after soaking TPS seeds in Petri-dishes.
2. Seedling vigour - scored at four weeks after sowing on the nursery trays, classified on a scale from 1=poorest to 9=most vigorous.
3. Survival rate - recorded at two weeks after transplanting in the field.
4. Vigour of plant canopy- measured at four weeks after transplanting or the emergence of tubers. Classified according to the scale: 1=poorest and 9=most vigorous.
5. Field resistance to late blight - measured during late August, i.e., at the end of rainy season. Classified on a scale from 1=poorest to 9=most resistant.
6. Maturity - recorded at two weeks before harvesting. Performance per family was rated on a scale: 1=earliest and 9=very late.
7. Uniformity - was evaluated on the basis of the following six characters: Flowering time, flower colour, plant type, plant height, tuber size and tuber skin colour. Performance per family was rated on a scale from 1=highly heterogeneous to 9=most uniform.

8. Selection rate - calculated on the basis of the number of clones suitable for breeding objectives.
9. Tuber number
10. Tuber yield (g/plant)
11. Marketable rate (% of 50g or more /tuber).

The characters mentioned from 9-11 were assessed at harvest. For this purpose, 30 random plants were chosen from each experiment unit, i.e., a total of 90 plants in each case.

12. Specific gravity - was calculated on the basis of weight of tubers in air/ (weight in air - weight in water). For the purpose of analysis, S.G value was transformed to (s.g.value - 1) x 10,000 .

The analyses of variance (ANOVA) were carried out for each of the following four traits: square root of tuber number, tuber yield, marketable rate and specific gravity according to a randomized block design with three replicates. Correlation between all traits were computed using the software Quatro pro 5.0. The analyses of other data were based on the mean values of progenies in each year. The general mean of each individual trait in the progenies was calculated on the basis of the progeny means of the parents involved. The general contribution of each parent- male or female- with respect to performance was partitioned. Combining ability analyses were based on an interaction model and an additive model by implementing the General Linear Models Procedure (SAS System, 1994).

5.3. Results

5.3.1 Investigation of seedling generation

In order to evaluate which types of seedling populations would be desirable in connection with TPS production or for analytic breeding, seeds from three different sources were grown and compared at Bashang (Hebei province) during 1993. These included the progenies from 4x-2x, 4x-4x and 4x selfings. Using all the three types of 4x progenies, 12 characters were compared starting from the stage of seed germination upto plant maturity together with some of the tuber characteristics (Table 5.1a). In the case of 4x-2x crosses, fourteen 4x female parents and twelve 2x pollen parents (2n-pollen producers) were used. In the resulting 4x progeny the contribution of the female and male parents was calculated separately (Table 5.1a). The number of 2n-pollen parents used for crossing with each 14 parents was not the same. In some cases, only one of the male parents was involved whereas in others as many as six male parents were tested. For estimating the female parental contribution to the 4x progeny, comparisons were made regardless of the number of crosses that were available.

The primary results of the parental general contribution to 4x-2x TPS progenies and comparison of the performance of progenies out of each parent showed that overall means in the progenies of Estima, Chippewa and Asterix produced a high germination rate; whereas Katahdin and Gineke had a low germination rate. For the 2n-pollen parents, progenies with CD1045 as male were quite outstanding; the rest were slightly low but was considerable. As to seedling vigour, progenies of Isola, Asterix, Escort, EC394 and Q9201-59 were highly acceptable, but Desiree and ED1022 gave the poorest. However, the survival rates of seedling populations were not consistent with a same trend as found after germination. Desiree and ED1022 produced a high survival rate for the progenies. Parental effects on plant vigour were similar except Estima, Premiere and ED1022 which were relatively low. Uniformity of progenies from parents was quite various. The progenies of Merrimack, Desiree, CD1045 and ED1045 performed well in uniformity of flowering time and flower colour. ED1022 contributed greatly to progenies in the uniformity of flower colour. At the same time, uniformity of plant canopy was evaluated among progenies, Merrimack, Desiree and Estima as female and CD1045, Q9201-59, ED1022 and EC394 as males gave positive effects on uniformity of tuber size in seedling generation. Generally speaking, there were not much differences among progenies except Desiree which yielded highly segregating progenies (score=5.7). For the uniformity of tuber skin colour, progenies of Gineke were unusually low, the rest of the parents were better and more or less similar (Table 5.1a). Isola and Q9201-59 produced relatively high tuber numbers and low yields (Table 5.1b). However, EC394 and CD1045 produced high tuber numbers and quite high tuber yield per plant. For late blight resistance of all these parents, their performances were satisfactory except for Katahdin, Premiere and ED1045. All values of parents with regard to maturity were more than 7 which means that parent contributions were influenced in the direction of lateness. Even ED1022, ED1045, Premiere, Estima and Escort themselves were relatively early. Finally the diploids Q9201-59, ED1045, ED1022 and CE10 and the tetraploids Premiere, Katahdin and Asterix showed higher specific gravity in their progenies. After the scores of seedling vigour, plant vigour, uniformity of flowering time, flower colour plant canopy, tuber size and tuber skin colour; and late blight resistance were pooled together and compared, it was shown that Merrimack was the highest in general (68) while Katahdin gave the lowest scores (54). For diploid 2n-pollen genotypes, CD1045 was the best with a sum score of 62.7. EC394, Q9201-59 and CE10 were acceptable with slightly lower sum scores.

Besides the above mentioned general characteristics, five important agronomic characters, viz., number of tubers per plant, yield per plant in grams, late blight field resistance, marketable rate and specific gravity of tubers were estimated in all the three types of 4x-progeny populations (Table 5.1b). The overall mean of the number of tubers per plant in the progenies of 4x-2x crosses was higher than those of 4x-4x crosses and 4x-selfings. Marked differences in tuber yield were observed in the three types of populations, the lowest being the 4x-selfed progenies. There were no notable differences in the overall means of plant maturity among the three populations. The values of specific gravity showed considerable variation among the individual plants of the same combinations. Thus, in the progenies of 4x-2x crosses, the specific gravity ranged from 784 to 1017 among individual parent means. In general, there were advantages in the overall means between progenies of 4x-2x and 4x-4x crosses.

In all, CD1045, EC394 and Q9201-59 were better 2n-pollen genotypes for TPS progenitors. Escort, Estima, Premiere and Merrimack were suitable for 4x-2x TPS production as evaluated at seedling generation.

5.3.2 Evaluation of clonal generation

The tubers collected from the seedlings of 1993 were planted in the field during 1994. These populations, the so-called clonal generation, were evaluated for 15 characteristics and the data are presented in Tables 5.2a and 5.2b. For the sake of comparisons, three locally tested 4x-clones, W2, Mira and Spunta, were used as female parents to cross with diploid clones, D-2-1, D-6-1 and D-7-1.

Evaluation of the most important agronomic traits indicated that the progenies of Katahdin, W2 and Eba; Q9201-59, D-2-1, D-7-1, CE1063 and ED1022 gave higher values. R16B-19, Estima, Chippewa, Merrimack and Gineke had lower effects on plant vigour. CD1045 and ED1045 had a less vigorous canopy. For field resistance to late blight, D-2-1, D-7-1 and Asterix possessed higher scores but progenies of Isola, Estima, R16B-19, Premiere, Escort and ED1022, ED1045 and EC394 showed low levels of resistance (Table 5.2a). Escort, Estima and Merrimack along with ED1022, ED1045, CE1063 shifted the progenies towards earliness. However, R16B-19, Asterix, and W2 along with 2n-pollen parents such as, D-2-1, D-7-1, EC394 gave rise to very late progenies. In the clonal generation, the parental contribution to the progenies was quite different from those in the seedling generation, which gave the chance for selection of TPS progenitors at both seedling and clonal stage. For TPS utilization, progenies have to be highly satisfactory in performance not only in seedling generation but also in the clonal generation.

Uniformity of canopy types was sufficiently high in general, especially the progenies of Chippewa, Merrimack, W2, ED1022, D-2-1, D-6-1, D-7-1 and CE10. However, Isola, CE1063 and ED1045 produced highly segregating progenies. Flowering time was quite uniform in most progenies except those from R16B-19 and CD1045. Flower colour in the populations was poor in uniformity, e.g., Asterix, Gineke, CD1045 and ED1045 but the locally selected three clones

(D-2-1, D-6-1 and D-7-1) and Q9201-59, ED1022 and EC394 were acceptable. Economically the most important traits are uniformity of tuber size and tuber skin colour. The results showed that uniformity of tuber size reached a relatively high level and in most cases the scores were 8.0 or more, except Gineke (6.5) and D-2-1 (6.5). Tuber skin colour was found to be the most convenient phenotypic character which was helpful for comparison with the uniformity of the seed tuber propagated cultivars. In the set of 4x-2x crosses there were indications that skin colour was relatively stable in the progenies of ED1022, D-6-1, CD1045, EC394, D-2-1 and D-7-1; along with Chippewa, Escort, Katahdin, Eba, Estima, and W2 (Table 5.2a). In the clonal generation, the breeder's preference score (general score) to each combination was assessed. The pooled data were presented in the form of general scores for each parent. From the Table 5.2a, Escort, W2, Asterix, D-6-1, EC394, ED1022, D-2-1 and CD1045 were suitable for TPS progenitors and also for analytic breeding when the selection rates were considered. The sum value of all records in a grade of 1-9 in descending order suggested that W2, Katahdin, Eba and Escort, along with three locally selected diploid clones, ED1022, Q9201-59 and EC394 were better.

Evaluation of yield components indicated that tuber numbers in clonal generation were increased as compared with the seedling generation. R16B-19 produced the highest tuber number in the progenies combined with a relatively high yield and similar observations were made for Katahdin and CD1045, EC394. Yield values were not so high as expected due to very late maturity of most progenies. The marketable rates were acceptable (72-87%) and showed less variation because the tubers were not fully mature. As TPS varieties are potentially important for starch processing, the starch content is an important consideration. In this context, all progenies had relatively high specific gravity (>850 or 1.0850 which has been required from processing sectors). Especially D-6-1, Q9201-59, D-2-1, D-7-1 and ED1045 along with W2, Mira, Spunta, Eba, Merrimack and Premiere can be used as parents for producing offsprings with high dry matter contents (S.G. from 1.0903 to 1.1070).

A comparison of the overall means of the general characteristics (plant vigour, uniformity) of the three types of progenies in the clonal generation revealed that the trend was comparable to the scores in the seedling generation and 4x-2x progenies had advantages over others in most cases (Table 5.2a). However, there were notable differences with respect to the number of tubers per plant and tuber yield per plant (Table 5.2b) which was expected.

For a more critical comparison of the four main agronomic characters (number of tubers per plant, yield per plant, marketable rate and specific gravity), analysis of variance was carried out for the data of both years (seedling and clonal generation). The ANOVA table is presented in Table 5.3. There were significant differences ($p<0.01$) between seedling and clonal generations with respect to tuber number and tuber yield. The contribution of the female parents (4x cultivars) was significant for tuber yield, marketable rate and specific gravity but not for tuber number. There were also significant differences among males for tuber number, tuber yield but not for marketable rate and specific gravity. This means, there is potential for selection of 2n-pollen parents in order to influence number and yield of tubers.

5.3.3 Correlation of 14 important traits for analytic breeding and TPS progenitors

Of the three types of populations that were investigated, none of the progenies showed uniformity for all or most of the characters analysed. In order to establish where there were any relationships between some of the characters studied, correlations between the progeny means of 14 characters in both seedling and clonal generations were calculated. Table 5.4 indicated that there was a significant correlation between the yield and the number of tubers, but this was not so in the case of seedling generation. The tuber yield was negatively correlated with (late) maturity in the seedling generation. This means that selection for earliness might be an important requirement in the selection of TPS utilization in view of the fact that transplanting seedlings delays maturity. In the case of the clonal generation, however, there was a slight positive correlation with lateness. Furthermore, tuber yield was negatively correlated with plant vigour in both seedling and clonal generations. With regard to specific gravity of tubers, it was not correlated either with maturity or tuber number but it was significantly negatively correlated ($p<0.05$) with yield in both seedling and clonal generations.

Late blight resistance was highly dependent on maturity and plant vigour in the clonal generation, but such dependence was absent in the seedling generation. This indicated that evaluation for late blight resistance in the seedling generation was not reliable.

5.3.4 Correlation between seedling generation and clonal generation

Besides examining the correlation between traits within the seedling and clonal generations of 4x-2x progeny (Table 5.4), the correlation between the two types of generation were also calculated for 11 characters (Table 5.5). There was a significant correlation between two generations in the plant vigour, tuber yield, specific gravity and uniformity of tuber skin colour. Such correlations were absent for other characters. At least for those traits that were positively correlated between the populations at seedling and clonal stage, it might be possible to select desirable combinations and genotypes in the early (seedling) generation.

5.3.5 Combining ability analyses for agronomic traits in 4x-2x crosses

Based on the data of four agronomic characters, viz., tuber number, tuber yield, marketable rate and specific gravity, of the progenies in the 4x-2x crosses, both general and specific combining abilities were calculated (Table 5.6 and 5.7). In order to determine general combining ability, 14 tetraploid female parents (mostly cultivars) and nine diploid genotypes were included (Table 5.6). There were notable differences between both male and female parents for the four traits considered. As female parents, the cultivars Escort, Desiree, Katahdin and Premiere showed high GCA for tuber yield as well as marketable rate. Among these only Premiere showed high GCA for specific gravity but not the other two. Among the nine diploid pollen parents these were considerably different. The notable genotypes among these were BE62, CD1045 and EC394 which showed high GCA for tuber yield. Two genotypes, CB122 and ED1045 showed relatively high GCA for specific gravity.

Specific combining ability was estimated in the 4x-2x progenies of six tetraploids and seven diploids in 22 combinations (Table 5.7). For tuber yield, high values of SCA were observed in the case of Asterix - CD1045, Asterix-ED1045, Desiree-CD1045 and Escort-EC394. High SCA for specific gravity was, however, observed in different combinations: Asterix-CE10, Desiree-CE1063, Eba-CD1045 and Escort-ED1045.

In all Escort-EC394, Escort-ED1022, Estima-CD1045, W2 x D-6-1, Asterix-ED1045, Desiree-CE1063, Premiere-EC394 and Asterix-Q9201-12 were recommended combinations for the TPS production.

5.4. Discussion

Whether it is for TPS production or for analytic breeding, there are many possibilities for producing sexual progenies. The choice of the method, however, largely depends on the purpose for which the progenies are used. If the aim is TPS production, then the emphasis will be to select parents that can give rise to highly vigorous, uniform progenies and produce high yields of agronomically acceptable tubers. This obviously requires the methods of improving the level of whole populations. On the other hand, if the aim is analytic breeding, the approach would be to produce sexual progeny that is suitable for a most efficient selection of a few genotypes with a desirable combination of agronomic characters. Such selected progenies should have the potential of becoming cultivars that can be propagated vegetatively.

Of various approaches suggested for both TPS production and analytic breeding (Hermsen, 1983; Rose, 1986; Clulow and Bradshaw, 1994; Clulow et al., 1995), three types of progenies derived from 4x-2x, 4x-4x and 4x selfings have been tested in the present investigation. Of these, the progenies of 4x-selfings as such are not attractive because they fall short of the expectations for several characters due to inbreeding depression. Such inbred lines are less attractive as parents because of their increased degree of sterility (Atlin, 1985; Kidane-Mariam et al., 1985; Hermsen, 1983). In view of these considerations, the selfed progeny of tetraploids are not attractive for TPS production although they might be useful as parents.

The progenies both from 4x-2x and 4x-4x crosses appear to be attractive for TPS production. Although 4x-4x crosses have been mostly used in the past for TPS production (Li et al., 1979; Qu et al., 1988; Upadhya, 1988), 4x-2x crosses are attractive for several reasons: 1) it is easy to genetically diversify the parents at the diploid level and select the proper parents; 2) quantitatively inherited characters and combining qualitatively inherited characters, especially disease resistances (chapter 4) can be more effectively transferred from diploid male parents to the tetraploid progenies; 3) favourable inter- and intragenic interactions (heterozygosity and epistatis) can be more conveniently transferred from diploid parents that produce FDR gametes; 4) selection of agronomically acceptable diploid clones that also form 2n-pollen is feasible (Chapter 2 and 3); and 5) highly vigorous progenies that produced uniform and high yields in a range of environments have been reported for families derived from 4x-2x crosses (Mascaso-Khwaja and Peloquin, 1983; Kidane-Mariam et al., 1985; Concilio and Peloquin, 1988; Peloquin et al., 1989; Hutten, 1994).

In view of the above attractive features, the large number of progenies from 4x-2x combinations, evaluated both in seedling and clonal generations, reveal certain interesting features. The general performance of all the progeny in seedling generation was inferior to that of clonal generation. However, a generalization cannot be made for all the characters investigated (Table 5.1 and 5.2). There were significant differences between the generations for the number of tubers and yield. Among the 14 female parents tested, there were significant differences between the genotypes for yield, marketable rate and specific gravity. Similarly, among males (2n-pollen producers) there were significant differences for the four important agronomic characters tested, viz., number of tubers, yield per plant, marketable rate and specific gravity (Table 5.3). Some of the individual characters were fairly uniform in certain progenies (Table 5.1 and 5.2), but this does not necessarily mean that they were uniform for all the characters studied. These results are in agreement with those of Tai and De Jong (1991) where there was no correlation between the uniformity for tuber colour and tuber shape in sexual progenies grown for TPS. Such results highlight the difficulties of producing progeny that show uniformity for several traits.

A comparison of general and specific combining abilities (GCA and SCA) for both female and male parents (Table 5.6 and 5.7) provide certain useful indications regarding the parental contributions. For tuber yields Escort and Premiere show high general combining abilities, but only the latter causes a high value for specific gravity. Among the pollen parents, although there are genotypes such as BE62, CD1045 and EC394, that show high values for tuber yields, all of them have negative values for specific gravity. This was not unexpected because the parental genotypes were not specifically selected to integrate these characters in the progenies. The only genotype, CB122, which has high GCA for specific gravity has a negative GCA for tuber yield. In view of GCA comparisons for some of the main agronomic traits, it appears, that the improvement of populations for several characters at a time is a difficult task.

As regards specific combining ability, high values in four traits were observed for the combinations Asterix-CD1045, Desiree-CE1063 Premiere-EC394 and Estima-CD1045. Among these, only the combination Desiree-CE1063 showed both positive values for marketable rate and high value for specific gravity. This combination has been recommended for trials by the farmers for TPS utilization (Table 5.7).

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Table 5.1a Contribution of 4x - and 2x parents based on means of 4x-progenies (seedling generation) evaluated at Bashang, Hebei province of China in 1993 for ten general traits.

Parent	No.cross	Germ-nation rate	Seedling Vigor	Survi. val rate	Plant Vigor	Uniformity flowering time	flower color	plant canopy	tuber size	tuber skin	Sum (a,b,c,d,,f,g,h,)
(I) 4x-2x											
Female			a		b	c	d	e	f	g	
Asterix	4	92.7	8.5	85.5	9	7	6.8	7.7	7.5	7.3	62.1
Chippewa	1	95.7	7	64	9	7	7	7	8	9	61
Desiree	4	80.4	6	92.9	7.5	8.5	7.5	8	5.7	7	58.7
Eba	3	82.1	7.7	83.4	8.7	6.3	6.7	7.7	8.7	8.7	63.2
Escort	5	74.3	8.2	90.8	8	7	7	7.4	9	8.8	63.4
Estima	3	96.9	7.7	85.7	6.3	7	7	8.3	9	8.3	61.3
Gineke	6	65.7	7.7	71.4	8.3	6	6	5.3	7.7	5	55
Isola	1	89.4	9	100	9	5	5	7	9	7	60
Katahdin	1	55	7	74.3	9	5	7	5	7	9	54
Merrimack	1	78.9	7	83.6	9	9	7	9	9	9	68
Premiere	3	88.5	7.7	75.1	6.3	5.7	5.7	5.7	9	8.3	54.7
Q9201-12	1	89	7	83	8	5	6	5	7	7	52
R16B-19	1	75	7	73.3	7	7	7	7	7	7	58
Overall mean (4x)		82.3	7.54	81.8	8.09	6.61	6.59	6.9	7.97	7.81	59.4
Male											
BE62	1	92	9	89	9	7	5	7	7	5	58
CB122	1	80	7	83	7	7	7	5	7	3	52
CB32	1	65.5	7	70.2	9	7	7	7	7	5	58
CD1045	6	92.6	7.7	82.2	7.5	7.5	7.2	8.2	8.3	7.5	62.7
CE10	6	78.3	7.5	78.6	8.5	6.3	6.3	6.8	8.3	8.5	60
CE1063	3	70.5	7	92.1	7.7	6.3	5.7	5.7	8.7	7.7	56.5
EC394	4	86.2	8.5	85.2	8	6.2	6.5	7.5	8.7	7.5	61.2
ED1022	3	87.4	6.3	100	5.7	7	7.7	7.7	7	8.7	57.8
ED1045	5	81.1	7.8	80.7	8	7.2	6.2	7	7.4	8.8	59.2
FB122	1	76	7	75	9	7	7	5	9	5	58
FB155	1	76.5	9	75.8	9	5	7	5	7	3	54
Q9201-59	1	86.9	8	85.3	9	6	6	8	8	8	61
Overall mean (2x)		81.1	7.65	84.5	8.14	6.64	6.52	6.57	7.86	6.5	58.3
Overall mean											
(i)4x-2x	34	83.5	7.6	84.6	8	6.6	6.4	7	8	7.5	58.2
(ii)4x-4x	5	59	7.4	77.4	8.2	6.2	6.2	6.2	8.6	7	57.6
(iii)4x⊕	4	62.6	5	70.5	7	6.8	6.8	6.8	8.8	8	56

Parents involved in 4x-4x crosses are Asterix, Eba, Chippewa, Katahdin, Merrimack and Q9201-12.
Parents involved in 4x selfings are Asterix, Kondor, Katahdin and Desiree.

Table 5.1b. Contribution of 4x - and 2x parents based on means of 4x-progenies (seedling generation) evaluated at Bashang, Hebei province of China in 1993 for five agronomic traits.

Parents	No.Cross	No. tuber (per plant)	Yield (g/pl)	LB resist. in field	Maturity	Specific gravity*
(I) 4x-2x						
Female				h		
Asterix	4	5.8	134	8.3	9	954*
Chippewa	1	5.6	114	7	9	914
Desiree	4	5.8	154	8.5	9	927
Eba	3	6.8	122	8.7	9	1004**
Escort	5	6.8	183	8	7.4	947
Estima	3	5.4	177	7.7	7.3	866
Gineke	6	5.3	134	9	8.7	909
Isola	1	7.7	109	9	9	813
Katahdin	1	5.1	158	5	9	989*
Merrimack	1	5.7	140	9	9	949
Premiere	3	5.4	185	6.3	7.3	978*
Q9201-12	1	6.5	145	7	8	911
R16B-19	1	3.8	158	9	9	894
Overall mean (4x)		5.77	147.3	7.96	8.56	928.7
Male						
BE62	1	5.5	116	9	9	929
CB122	1	4.8	99	9	9	885
CB32	1	4.1	77	9	7	784
CD1045	6	6.5	177	8.8	8.3	907
CE10	6	5.2	126	7.8	8.7	979*
CE1063	4	5.5	155	7.7	9	908
EC394	4	7.6	175	8.3	8.8	933
ED1022	3	5.8	158	7.7	7	956*
ED1045	5	5	152	6.8	7.6	982*
FB122	1	5.3	121	9	9	883
FB155	1	7.6	171	9	9	884
Q9201-59	1	7.3	103	8	9	1017**
Overall mean (2x)		5.85	119.4	8.34	8.63	920.8
Overall mean						
(I) 4x-2x	34	5.9	151	7.1	8.3	933 (784-1086)
(ii) 4x-4x	5	4.9	122	7.8	8.2	961 (919-994)
(iii) 4x⊕	4	4.9	81.5	7	8.5	930 (917-944)

Parents involved in 4x-4x crosses are Asterix, Eba, Chippewa, Katahdin, Merrimack and Q9201-12.
Parents involved in 4x selfings are Asterix, Kondor, Katahdin and Desiree.

Table 5.2a Contribution of 4x - and 2x parents based on means of 4x-progenies (clonal generation) evaluated at Bashang, Hebei province of China in 1994 for eleven traits.

Parent	No.cross	Plant Vigor	Uniformity Canopy						LB resist.	Mature	Selection rate	General score	Sum of (a,b,c,d,e,f,g,h)
			Canopy	Height	flower color	flower time	tuber size	tuber skin					
(I) 4x-2x													
Female		a	b	c	d	e	f	g	h				
Asterix	4	7	7.2	6.2	7.5	5.8	8.2	7.2	7.2	8.5	5.3	8.5	56.3
Chippewa	1	6	9	7	9	7	7	9	7	7	0.7	8	61
Desiree	4	7	7.2	7.8	7.2	7	8.2	7.5	7	7	2.0	7.8	58.9
Eba	3	7.7	8	7	8.3	7.3	8.7	8.7	7	7.3	1.4	7.7	62.7
Escort	5	7.2	7.5	6.5	7.8	7.6	8.8	9	5.6	5	5.8	9*	60
Estima	3	5.7	7.7	7	7	6.7	8	8.7	4.7	6	2.3	8.3	55.5
Gineke	6	6.5	8.1	6.7	7.7	6.1	6.5	5.9	6.9	7	1.9	6.5	54.4
Isola	1	7	5	5	7	7	7	8	3	3	0	7	49
Katahdin	1	8	8	7	9	7	8	9	7	7	3.1	8	63
Merrimack	1	6	9	8	7	7	8	8	7	6	4.8	8	60
Premiere	3	7	8.2	7	7.5	7.7	8.2	8.2	5.2	7	1.8	8.2	59
Q9201-12	1	5	5	5	7	7	7	7	7	7	0	7	50
R16B-19	1	5	7	7	5	7	7	8	5	9	0	7	51
W2(CK)	2	8	9	9	9	9	8.5	8.5	7	8	2.5	9*	68
Mira(CK)	1	9	9	9	9	9	7	7	9	9	0	7	68
Spunta(CK)	2	7	9	9	9	9	9	9	9	9	0	9	70
Overall mean(4x)		6.65	7.54	6.90	7.58	7.08	7.77	8.05	6.19	6.77	2.26	7.83	57.75
Male													
BE62	1	9	9	5	9	7	7	5	7	9	0.8	7	58
CB122	1	1	7	5	5	3	5	3	5	7	0	5	36
CB32	1	9	7	9	7	7	9	9	5	9	3.3	8	62
CD1045	6	5.3	7.3	6.3	6.7	6.3	8.1	8.7	6.1	7.3	1.7	8.1	54.8
CE10	6	7.3	8	7	7.3	7	7.9	8.3	7	7.6	2.4	7.3	59.8
CE1063	4	7.7	6	5.7	7.3	7	8.3	7.3	7	6.3	4	8	56.3
D-2-1(CK)	2	9	9	9	7	9	6.5	8.5	8	9	4.1	8.5	66
D-6-1(CK)	1	7	9	9	9	9	9	9	7	7	5	9	68
D-7-1(CK)	2	8	9	9	9	9	8.5	8.5	8	9	0	8	69
EC394	4	7.2	7.8	7.6	8.2	8	8.4	8.6	5.6	7.8	4.2	8.8*	61.4
ED1045	5	6.6	6.8	5.2	7	6.2	8	7.6	5.6	6	2.8	7.8	53
ED1022	3	7.7	9	9	7.7	8.3	8.3	9	5	4.3	4.8	8.7*	64
FB122	1	5	8	5	9	7	5	5	9	7	1.6	5	53
FB155	1	7	9	9	9	7	5	5	7	9	0.5	7	58
Q9201-59	1	9	7	8	8	7	9	8	7	7	0.8	8	63
Overall mean(2x)		7.48	7.89	7.58	7.72	7.68	8.20	8.35	6.63	7.13	3.70	8.22	61.53
Overall mean													
(I) 4x-2x	39	7	7.7	7	7.7	7.1	8.1	8.3	6.5	7.1	2.7	8.3	59.4
(ii) 4x-4x	4	7.8	8	7.5	7	6.8	7.3	6.8	5.8	8.8	5.3	7.5	56.5
(iii) 4x⊕	4	6	8	6.5	7	7	7.8	7.5	6	8	4.7	7.8	55.3

Parents involved in 4x-4x crosses are Asterix, Eba, Chippewa, Katahdin, Merrimack and Q9201-12.
Parents involved in 4x selfings are Asterix, Kondor, Katahdin and Desiree.

Table 5.2b. Contribution of 4x - and 2x parents based on means of 4x progenies (clonal generation) evaluated at Bashang, Hebei province of China in 1994 for four agronomic traits.

Parent	No.cross	No.tuber	Yield (g/pl)	Mark. rate (%)	Specific gravity
(i) 4x-2x					
Female					
Asterix	4	8.2	376	81.2	876
Chippewa	1	7.3	342	81.9	835
Desiree	4	7.8	343	82.3	863
Eba	3	8.6	336	78.6	933*
Escort	5	8.3	385	84.8	863
Estima	3	8.6	347	81.8	819
Gineke	6	9.3	346	77.8	877
Isola	1	7.6	233	71.7	857
Katahdin	1	9	419	87.4	777
Merrimack	1	8.1	367	79.3	905*
Premiere	3	9.8	380	79.3	904*
Q9201-12	1	6.3	233	75.2	889
R16B-19	1	10.3	397	81.4	844
W2(CK)	2	7.3	298	79.9	1030**
Mira(CK)	1	7	213	60.9	1017**
Spunta(CK)	2	5.5	277	78.5	977
Overall mean (4x)		8.08	330.5	78.6	889.6
Male					
BE62	1	9.9	397	80.7	823
CB122	1	8.4	247	75.7	978
CB32	1	10.7	367	82.7	770
CD1045	6	9.9	412	82.3	847
CE10	6	8.2	354	86.1	861
CE1063	4	9.2	331	81.7	875
D-2-1(CK)	2	8.9	275	67.6	997**
D-6-1(CK)	1	9	327	79.6	1070**
D-7-1(CK)	2	5.5	270	80.2	989*
EC394	4	8	381	79.6	874
ED1022	3	7	313	83.1	824
ED1045	5	8.2	346	81.4	903*
FB122	1	8.5	333	73.4	892
FB155	1	11.1	410	73.4	923*
Q9201-59	1	7.4	312	78.6	1003**
Overall mean (2x)		8.7	338.4	79.1	908.8
Overall mean					
(I) 4x-2x	39	8.6	346	80	889(770-1070)
(ii) 4x-4x	4	10	466.5	81.2	887(863-925)
(iii) 4x⊕	4	6.5	317	82.5	838(718-917)

Parents involved in 4x-4x crosses are Asterix, Eba, Chippewa, Katahdin, Merrimack and Q9201-12.
Parents involved in 4x selfings are Asterix, Kondor, Katahdin and Desiree.
* and ** mean good and very good.

Table 5.3 Analyses of variance of four traits in the progenies of 4x-2x crosses.

Source	Df	Mean squares			
		√ (No.tuber)	yield/plant	Mark. rate	Specific gravity
Years	1	14.9770**	2428304.16**	–	–
Block (year)	4	0.0708	6449.72	–	–
Female	12	0.2348	18496.20**	86.6456**	5388.61**
Male	11	0.4099**	16069.35**	49.9894**	10781.95**
Female x Male	10	0.2697*	12554.51**	56.9327**	7544.11**
Error		0.1411 (165)	4010.47 (165)	16.6111 (68)	2219.049 (65)

* Significant difference at a level of $p = 0.05$, ** Significant difference at a level of $p = 0.01$.
Data in ( ) is freedom for each parameter.

Table 5.4 Correlationship of 14 trait parameters for selection of TPS progenitors and parents in analytic breeding.

Dependent trait(Y)	Independent trait(X)	Regression formula	R squared value	
Tuber yield(g/pl)	tuber number	$Y=147.2-0.55X\pm28.6$	0.0004	S
		$Y=125.9+26.60X\pm40.5$	0.3458**	C
	maturity	$Y=338.9-23.0X\pm23.5$	0.3289**	S
		$Y=294.9+7.37X\pm48.9$	0.0451	C
	plant vigor	$Y=249.9-13.33X\pm25.3$	0.2212*	S
		$Y=429.6-11.71X\pm48.5$	0.0597	C
Specific gravity	maturity	$Y=978.8-4.76X\pm47.9$	0.0050	S
		$Y=804.9+12.66X\pm69.6$	0.0644	C
	tuber number	$Y=953.9-2.67X\pm47.9$	0.0030	S
		$Y=981.6-10.58X\pm70.9$	0.0265	C
	tuber yield	$Y=949.7-0.08X\pm48.0$	0.0050	S
		$Y=1108.5-0.62X\pm64.9$	0.1855*	C
	marketable rate	$Y=886.9+0.02X\pm66.2$	0.1544*	C
	plant vigor	$Y=872.0+8.37X\pm47.3$	0.0311	S
		$Y=637.6+35.2X\pm61.3$	0.2751**	C
Late blight field resistance	maturity	$Y=4.69+0.37X\pm1.0$	0.0655	S
		$Y=2.64+0.53X\pm0.88$	0.4349**	C
	plant vigor	$Y=6.96+0.11X\pm1.03$	0.0118	S
		$Y=2.84+0.50X\pm1.04$	0.2003*	C
	seedling vigor	$Y=5.68+0.29X\pm1.0$	0.0644	S
Marketable rate	plant vigor	$Y=86.2-0.78X\pm4.17$	0.0372	C
	tuber yield	$Y=60.5+0.06X\pm3.09$	0.4696**	C
	tuber number	$Y=79.7+0.11X\pm4.24$	0.0009	C
Uniform tuber size	maturity	$Y=10.97-0.36X\pm0.96$	0.0682	S
		$Y=8.37-0.06X\pm0.73$	0.0133	C
	tuber yield	$Y=7.40+0.0034X\pm0.99$	0.0098	S
		$Y=8.01-0.0001X\pm0.73$	0.0001	C
	tuber number	$Y=7.70+0.03X\pm0.99$	0.0010	S
		$Y=9.26-0.16X\pm0.71$	0.0566	C
Maturity	uniform flower color	$Y=9.41-0.14X\pm0.71$	0.0173	S
		$Y=5.0+0.28X\pm1.42$	0.0311	C
Uniform plant type	uniform flower color	$Y=2.37+0.72X\pm0.93$	0.2116*	S
		$Y=3.25+0.62X\pm0.84$	0.3184**	C
Uniform plant height	uniform plant type	$Y=0.25+0.89X\pm0.72$	0.6135**	C
Uniform flower time	uniform flower color	$Y=0.54+0.93X\pm0.74$	0.4110**	S
		$Y=4.34+0.45X\pm0.84$	0.1984*	C
Uniform tuber skin	uniform flower color	$Y=4.79+0.47X\pm0.94$	0.0996	S
		$Y=5.07+0.42X\pm0.63$	0.2786**	C
Plant vigor	seedling vigor	$Y=4.56+0.46X\pm0.92$	0.1663*	S
Survival rate	seedling vigor	$Y=68.3+1.95X\pm9.04$	0.0366	S

Continue ......

General score	selection rate	$Y=7.56+0.16X\pm0.55$	0.2586**	C
	maturity	$Y=7.93+0.02X\pm0.64$	0.0015	C
	late blight field resis.	$Y=7.48+0.09X\pm0.63$	0.0271	C
	uniform tuber skin color	$Y=3.93+0.50X\pm0.52$	0.3445**	C
	uniform tuber size	$Y=4.13+0.49X\pm0.53$	0.3196**	C
	uniform flower color	$Y=5.29+0.38X\pm0.53$	0.2987**	C
	uniform flower time	$Y=5.77+0.30X\pm0.57$	0.1922*	C
	uniform plant height	$Y=6.19+0.26X\pm0.56$	0.2221*	C
	uniform plant type	$Y=6.05+0.26X\pm0.58$	0.1686*	C
	plant vigor	$Y=6.72+0.19X\pm0.61$	0.0947	C
	specific gravity	$Y=4.87+0.004X\pm0.58$	0.1613*	C
	marketable rate	$Y=7.19+0.01X\pm0.64$	0.0051	C
	tuber yield	$Y=7.61+0.001X\pm0.63$	0.0099	C
	tuber number	$Y=8.54-0.06X\pm0.63$	0.0105	C
	(tuber No+tb yield+mark. rate+S.G)	$Y=1.85+0.02X\pm0.55$	0.2496**	C
Selection rate	tuber number	$Y=3.50-0.04X\pm2.06$	0.0006	C
	tuber yield	$Y=0.04+0.009X\pm2.02$	0.0467	C
	marketable rate	$Y=-2.20+0.07X\pm2.05$	0.0185	C
	specific gravity	$Y=-3.45+0.01X\pm2.0$	0.0658	C
	plant vigor	$Y=-2.77+0.83X\pm1.87$	0.1771*	C
	maturity	$Y=3.46-0.05X\pm2.06$	0.0011	C
	uniform plant type	$Y=1.89+0.16X\pm2.06$	0.0062	C
	uniform plant height	$Y=0.48+0.37X\pm2.02$	0.0429	C
	uniform flower time	$Y=1.09+0.27X\pm2.05$	0.0147	C
	uniform flower color	$Y=2.26+0.12X\pm2.06$	0.0028	C
	uniform tuber size	$Y=-4.91+1.01X\pm1.93$	0.1281*	C
	uniform tuber skin	$Y=3.75-0.07X\pm2.06$	0.0007	C
	late blight field resis.	$Y=0.33+0.44X\pm2.0$	0.0615	C
	(tuber No+tb yield+mark. rate+S.G)	$Y=-14.48+0.013X\pm1.86$	0.1913*	C

S = seedling generation; C = clonal generation.

Table 5.5. Correlation of parent performances in seedling and clonal generation.

Dependent trait (Y) (data of 1994)	Independent trait (X) (data of 1993)	Regression formula	R squared value
Plant vigor		$Y=3.65+0.41X\pm0.91$	0.1716*
Uniformity of tuber skin color		$Y=3.49+0.58X\pm0.52$	0.5561**
Tuber yield		$Y=253.4+0.72X\pm42.28$	0.1905*
Specific gravity		$Y=456.9+0.44X\pm41.26$	0.2082*
Tuber number		$Y=10.36 - 0.34X\pm0.93$	0.1126
Late blight resistance		$Y=6.95 - 0.10X\pm1.13$	0.0083
Uniformity of flowering time		$Y=9.49 - 0.30X\pm0.80$	0.1190
Uniformity of flower color		$Y=8.70 - 0.26X\pm0.58$	0.0799
Uniformity of canopy		$Y=7.38+0.03X\pm0.96$	0.0011
Uniformity of tuber size		$Y=7.43+0.06X\pm0.67$	0.0069
Maturity		$Y=1.25+0.65X\pm1.33$	0.1093

Table 5.6 General combining ability of 13 tetraploid cultivars and 11 diploid genotypes for square root of tuber number,tuber yield, marketable rate and specific gravity based on evaluation of the progenies of 4x-2x crosses.

Parent	Square root of No. tuber	Tuber yield	Marketable rate	Specific gravity
4x				
Asterix	-0.03	-9.77	-0.27	21.80
Chippewa	-0.05	-4.78	0.15	-22.36
Desiree	-0.06	13.89	4.75	14.48
Eba	-0.01	-35.45	-0.04	20.16
Escort	0.16	47.03	6.53	6.90
Estima	-0.15	6.31	3.57	-47.68
Gineke	-0.19	-43.78	-4.59	-41.36
Isola	0.02	-118.58	-13.21	12.20
Katahdin	0.12	45.06	5.68	-84.36
Merrimack	0.12	-12.11	-1.92	29.98
Premiere	0.11	37.83	4.35	46.88
R16B-19	-0.35	7.80	3.84	2.00
Q9201-12	-	-	-6.55	19.64
2x				
BE62	0.16	41.90	5.17	-16.97
CB122	-0.03	-41.44	0.14	138.03
CB32	0.09	18.56	7.21	-70.64
CD1045	0.16	29.92	-2.56	-40.00
CE10	-0.17	-9.44	1.67	-12.31
CE1063	-0.13	-45.52	-3.64	-29.76
EC394	-0.01	31.70	3.76	-36.86
ED1022	-0.26	-42.85	-1.92	-33.62
ED1045	-0.22	-27.31	-2.08	16.99
FB122	0.04	12.89	-2.16	52.03
FB155	0.46	76.06	-2.13	82.69

Table 5.7 Specific combining ability effects for tuber number per plant, tuber yield per plant, marketable rate and specific gravity in 4x-2x progenies (the rest combinations are 0.0).

Pedigree	Tuber number	Tuber yield	Marketable rate	Specific gravity
Asterix - CD1045	0.32	45.96	-3.05	-39.13
Asterix - CE10	0.03	-14.34	1.46	41.18
Asterix - EC394	-0.28	-63.48	-3.53	-9.27
Asterix - ED1045	-0.08	31.86	5.14	7.22
Desiree - CD1045	0.06	-67.03	-5.47	-7.47
Desiree - CE1063	0.06	37.08	4.31	75.62
Desiree - ED1022	-0.13	15.74	0.36	-
Desiree - ED1045	0.02	14.20	0.82	-68.13
Eba - CD1045	0.03	-14.36	1.46	41.18
Eba - CE10	-0.03	-14.34	1.44	-41.18
Eba - Q9201-59	0.0	0.0	0.0	0.0
Escort - CD1045	-0.33	9.83	5.58	- 0.58
Escort - CE1063	-0.13	-48.06	-5.54	-47.80
Escort - EC394	0.24	45.72	1.99	-4.39
Escort - ED1022	0.12	-7.40	-0.83	14.37
Escort - ED1045	0.09	-0.11	-1.20	38.43
Estima - CD1045	-0.09	25.55	1.51	6.02
Estima - ED1022	0.01	-8.35	0.47	-14.36
Estima - ED1045	0.08	-17.22	-1.97	8.36
Premiere - CE1063	0.06	10.97	1.25	-27.78
Premiere - EC394	0.03	17.75	1.55	13.65
Premiere - ED1045	-0.09	-28.74	-2.78	14.14

Chapter 6

General discussion

In diploid potato, like in a large number of other plants (Harlan and de Wet, 1975), 2n-gametes occur frequently. In principle, the occurrence of 2n-eggs and 2n-pollen might be expected to be equally frequent, but critical estimation of their frequencies can be difficult and are not available. Generally, it is far more easy to detect 2n-pollen than 2n-eggs in diploid potato. This is because pollen grains can be easily monitored for their size by mounting a sample in a staining solution such as acetocarmine or lactophenol acid fuchsin. The presence of large pollen grains with four germpores together with smaller ones with three germpores is a reliable indication for the occurrence of 2n-pollen. It is, however, far more difficult to monitor the occurrence of 2n-eggs. Because it is not possible to directly sample the egg cells cytologically, an indirect method is required to detect 2n-egg formation. One convenient method is to make a 2x-4x cross, estimate the level of seed set and count the chromosome number in the progenies. The level of seed set in the 2x-4x cross can already give a good indication of 2n-eggs because, in the absence of 2n-eggs in the diploid parents, there will be hardly any seed set due to the so called "triploid block" (Marks, 1966). Generally, a higher rate of seed set in 2x-4x crosses can be used as a good criterion for the selection of diploid clones that produce 2n-eggs. In such cases, the presence of 4x individuals in the progenies has to be confirmed through chromosome counting or DNA measurements. Because of the difficulties associated with the selection of 2n-eggs, and also due to their gametic make-up being predominantly SDR (Jongedijk, 1985), it was more convenient to use 2n-pollen producing clones in the present investigation.

Whether it is for analytic breeding or for TPS cultivar production, selection of genotypes that produce high frequencies of 2n-pollen in various environments and high seed set in 4x-2x crosses is an important pre-requisite. In this connection, the well-known clones USW5293-3, USW5295-7, USW5337-3, USW7589-2 and 772102-37, which produced high frequencies of 2n-pollen in other parts of the world (Chapter 2), also proved to be successful in 2n-pollen formation in the environments of China. Although there was considerable variation for the frequencies of 2n-pollen in the progenies of their diallel combinations, it is certainly possible to select for clones that perform well in various environments. Looking to the combining ability, SCA contributed much more than GCA for 2n-pollen formation. This obviously indicated that there is a scope for selection of better genotypes for this character.

There are several wild *Solanum* species that produce 2n-pollen sporadically (Quinn et al., 1974; Den Nijs and Peloquin, 1977). Nevertheless, selection of diploid *S. tuberosum* is necessary in order to produce genotypes that produce constantly high frequencies of 2n-pollen in different environments. To this end, the repeated cycles of selection of genotypes for higher frequencies of 2n-pollen has been proven to be effective (Chapter 3). Clearly there was a fourfold increase in mean values of 2n-pollen in the high x high-backcross progenies (Table 3.2). This means, if suitable populations are selected, despite the complicated pattern of inheritance of 2n-gamete formation, it should be possible to select suitable clones through a combination of appropriate parents. The possibilities for combining desirable marker genes in order to select directly FDR 2n-pollen has been demonstrated by Bastiaanssen, et al., (in preparation).

Once the higher frequencies of 2n-pollen are achieved in the desirable clones, the question remains as to how effective is the transfer of characters through 2n-pollen. The transfer of bacterial wilt from the diploid genotype, ED1022, to 4x progenies in 4x-2x crosses (Chapter 4) illustrated the usefulness of this approach. From the pattern of inheritance of BW resistance in this investigation as well as the previous ones (see Chapter 4), it is clear that this character is quantitative and not efficiently transmitted to the offspring in the 4x-4x or 2x-2x crosses. It is difficult to transfer such a character to progenies. Because the parental gene combinations, including the inter- and intragenic interactions, are retained to a great extent in the FDR gametes such as the ones in ED1022, even the quantitative characters like BW resistance are amenable to transfer.

Besides single characters such as BW resistance, it is essential that a large number of traits, including agronomic ones, should be improved on a population basis if TPS breeding has to succeed. In this context, among the three types of crosses investigated, viz., 4x-2x, 4x-4x and 4x-selfings (Chapter 5), there were clear indications that the 4x-2x combinations were more attractive than others. Similar observations have also been reported in potato recently by others (Clulow et al., 1995). In view of this, the use of 2n-pollen offers the prospects of producing TPS cultivars by 4x-2x crosses. From the estimation of GCA and SCA for four agronomic characters in the progenies of 4x-2x crosses it is evident that it is possible to select both 4x and 2x parents with high GCA for some of the important traits. Thus, the 4x parents Premiere, Desiree and Escort were good female parents and FB155, FB122, CD1045, CB122 and EC394 were suitable 2x male parents for TPS production.

Some of the crucial requirements of TPS cultivars are that they give rise to a highly vigorous progeny possessing the ability to produce satisfactory yields of tubers that are uniform in skin colour and size. In order to meet these requirements, it is essential to select male parents that produce 2n-pollen exclusively through FDR enabling transfer of genetic uniformity through the male parent. Such a situation, however, is relatively rare. In almost all diploid genotypes used in this investigation, the 2n-pollen originates predominantly through fused spindle formation during anaphase II of microsporogenesis (Ramanna, 1979). However, it is by no means certain that this is the only mechanism that operates in all these diploid clones under diverse environmental conditions. It should be pointed out that other mechanisms, such as pc1(premature cytokinesis), pc2 and pc3 that can lead to SDR, have been detected in some of the well-known parental clones (Mok and Peloquin, 1974) used in the present investigation. This means, the occurrence of a mixture of both FDR and SDR mechanism in one and the same clone cannot be ruled out completely. In view of this, the lack of uniformity for some of the characters analysed (Chapter 5) in this investigation is not surprising. This means, if uniformity and vigour of the progeny are the main considerations, then it is important to select genotypes that produce exclusively FDR 2n-pollen.

In principle, it should be possible to select genotypes that produce 2n-pollen only through fused spindles. In these rare cases, however, it is theoretically possible that SDR can also occur incidentally or more frequently depending upon the environment. One method of producing exclusively FDR 2n-pollen is through the use of desynaptic mutants (Okwuagwu and Peloquin, 1981; Hermsen and Ramanna, 1981). In these mutants the homologous chromosomes fall apart resulting in the formation of univalents at metaphase I of microsporogenesis producing total sterility. When such desynaptic mutants produce 2n-pollen either through pseudohomoeotypic division or through fused spindles (Ramanna, 1983; Jongedijk and Ramanna, 1989), only then the fertile 2n-pollen is produced. The remarkable feature of desynaptic mutants is that the SDR pollen grains abort in them because of the imbalance of chromosome numbers resulting from abnormal separation during anaphase I.

The possibility of producing exclusively FDR pollen on a large scale using different genotypes of desynaptic clones of diploid potato was first demonstrated by Ramanna (1983). The potential of these clones for sexual polyploidization was established by Jongedijk et al., (1991a). Using such genotypes, Clulow et al. (1995) showed that desynapsis can assist the production of phenotypically uniform TPS progenies. Uniformity of the FDR pollen in these cases results from the highly reduced recombination prior to meiotic nuclear restitution as is evident from the segregation of isozyme and other genetic marker loci (Jongedijk et al., 1991b; Douches and Quiros, 1988). One difficulty of using desynaptic mutants is that a large majority of them are highly sterile. In spite of this, it is possible to select desynaptic genotypes that have desirable agronomic traits as well as produce 2n-pollen.

For the purpose of TPS cultivar production, three alternative approaches for developing male parents can be considered. First, diploid clones that have a tendency to produce 2n-pollen can be selected and desirable agronomic traits are added later on. Second, nuclear restitution mechanisms can be added into agronomically acceptable clones through conventional breeding. Third, recombinant DNA methods can be applied to introduce genes to modify meiosis in the agronomically acceptable clones which have been improved at diploid level. All these three approaches have certain advantages and drawbacks.

In principle, it is possible to select diploid populations that have proper alleles to produce high frequencies of 2n-pollen and also use them to get high rate of seed set from 4x-2x crosses (Chapter 2 and 3). If agronomic traits are to be incorporated into such genotypes, they might lead to complications because both 2n-gamete formation and agronomic traits are complex characters. Another bottle neck is that once the FDR mechanism in a selected clone is disrupted for any reason (by using as a parent) it can be difficult to reconstitute the same mechanism in the progeny. This difficulty also holds true for the second approach in which the FDR mechanisms has to be added into agronomically acceptable genotypes. In both approaches the difficulty of selecting for such a quantitative trait remains.

It is therefore worthwhile to consider an alternative approach of inducing FDR gametes in potato, namely by genetic manipulation. In principle, it should be possible to generate an FDR phenotype by eliminating gene functions that are essential for (1) initiation of meiotic recombination, and (2) the persistence of sister chromatid cohesion during meiosis I. In yeast, several genes that are essential for meiotic recombination have already been identified and cloned (e.g., Alani et al., 1990; Cao et al., 1990). The cDNA encoding the tomato homologue of one of these genes, *RAD51*, has already been isolated by heterologous screening, to yield *LeRAD51* (Offenberg et al., unpublished experiments). Furthermore, one yeast gene, *SPO13*, has been identified and cloned that is essential for sister chromatid cohesion (McCarrol and Esposito, 1994). Simultaneous elimination of the functions of a recombination gene and of *SPO13* yielded a synthetic FDR mutant of yeast (Klapholz and Esposito, 1985). Thus, by introducing the antisense forms of potato-homologues of such genes into the diploid potato through genetic transformation, it should be possible to induce FDR 2n-gametes in desirable genotypes. Even though the process of meiosis is a combination of many continuous events such as homologous chromosome pairing, chiasma formation, spindle formation and cytokinesis, among others, all these are amenable for genetic modifications. In view of this, the production of desirable diploid potato clones with FDR 2n-gametes through the use of recombinant DNA techniques might be achieved. In that event, such procedures of modification of meiosis can add a new dimension for the production of uniform and highly heterotic TPS cultivars in potato.

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General Summary

Among the most important world crops, the cultivated potato occupies the fourth place in terms of growing area, distribution and production. This crop was first introduced into Europe during the last quarter of the 16th century and, as a result of breeding and selection, the present day potato, *Solanum tuberosum L. subspp. tuberosum* has originated. Although this crop is well adapted to the Northern temperate climate with long day length conditions, its performance in the subtropical and tropical regions is relatively poor. In view of the potential of potato to produce high tuber yields, it is attractive to adapt this crop fully to more populous, poorer tropical and subtropical regions. Unlike in the temperate regions, it is more difficult to grow potato in tropics and subtropics. Among many difficulties, potato being vegetatively propagated, it is highly vulnerable to numerous pests and diseases. Especially, when tubers are infected with viruses they cannot be used as planting material because it can lead to serious reductions in yield.

One idea which has attracted much attention in recent years is to grow potato through true potato seeds (TPS). There are many advantages of using this method. First, many of the potato diseases, especially virus diseases, are not transmitted through TPS; second, it is exceptionally inexpensive to grow a crop through TPS and does not involve the risk of using expensive seed tubers; third, the peasant farmers can produce their own seed; and finally, considerable quantities of tubers which are otherwise used for planting can become available as food. There are, however, a number of disadvantages and problems to be solved before the technology of TPS can become satisfactory. Some of the problems are, 1) the yield from seedlings after transplanting are relatively low; 2) the harvest is not as uniform as from the clonal propagation; 3) new technology and agronomic practices require to be developed.

The first two of the above mentioned problems can be overcome to a great extent through the use of 2n-gametes to produce TPS varieties by 4x-2x crosses. In such crosses 4x progenies are expected because of a “triploid block”. It has been well established that when 2n-gametes originate through first division restitution (FDR), they retain the parental heterozygosity intact and also they are genetically more uniform. This obviously opens the way to produce TPS potato which can be highly heterozygous (having heterosis) and also phenotypically uniform. Experimental work on using 2n-gametes has indeed established that TPS can be produced effectively through the process of sexual polyploidization, i.e., through the use of 2n-pollen.

Despite many previous attempts to grow potato through TPS in various countries, the first notable success was achieved in China. Since there are different types of agroclimatic regions in China where potato is being grown,

there is potential to develop potato cultivars that suit traditional methods as well as through the use of TPS. To this end, it was felt necessary to explore the possibilities of developing both TPS cultivars as well as traditional cultivars through analytic breeding. For this purpose, it was appropriate to utilize the plant materials developed at the Department of Plant Breeding, WAU, Wageningen, because of the availability of basic information. After setting up the experiments in Wageningen during the summer of 1992, the plant materials were grown and investigated in China during 1993 and 1994.

In the first place, a comparison was made of the frequencies of 2n-pollen production in a different environment using the progeny of diallel crosses between five well-known diploid clones, A (USW5293-3), B (USW5295-7), C (USW5337-3), D (USW7589-2) and E (772101-37). In addition, some of these clones (A, C and E) were crossed with three clones of *Solanum phureja* (IvP35, IvP48 and IvP101) almost not producing 2n-pollen. The frequencies of 2n-pollen were estimated in the progenies. A notable conclusion was that the frequencies of 2n-pollen in selected clones were relatively stable. There was variation for the frequencies of 2n-pollen among the families, and specific combining ability (SCA) contributed more to this variation than the general combining ability (GCA).

In order to gain an insight into the possibilities of increasing the frequencies of 2n-pollen, crosses were made between different types of clones with the following four types of combinations: low x low (low frequency of 2n-pollen), low x high, high x high and high x high- backcross. A total of 3971 plants from 36 combinations were evaluated for the frequencies of 2n-pollen. Looking to the segregation patterns for 2n-pollen formation, it was evident that this character has a complex type of inheritance but that it is not monogenic. It was evident that at population level through repeated cycles of selection for 2n-pollen a four-fold increase could be achieved in the case of high x high-backcross combinations.

Although increase of the frequencies of 2n-pollen was one important aim, it was also necessary to established that 2n-pollen can be effective for transferring useful characters from a selected male parent to the sexual polyploid progeny. In order to prove this, an attempt was made to transfer resistance against bacterial wilt (BW) of potato caused by *Pseudomonas solanacearum*. For this purpose, several diploid and tetraploid genotypes were screened for BW resistance and some resistant genotypes were selected. From the pattern of inheritance, it was evident that BW resistance was a quantitative character. The diploid genotypes which showed high resistance levels against BW and which also produced 2n-pollen were crossed in different ploidy combinations such as 4x-2x and 2x-2x. They were compared with 4x-4x crosses. It was demonstrated that transferring of resistance to BW was more effective in 4x-2x combinations. This obviously means that the use of 2n-gametes is effective in transferring a quantitative character like BW resistance to 4x progenies.

With a view to evaluate the optimal breeding approach for the production of TPS varieties, three different types of progenies were evaluated for about 15 characteristics. These progenies were derived from 4x-2x, 4x-4x and 4x-selfings. The performance of these progenies was evaluated in both the seedling generation and the clonal generation. Among the 15 characteristics studied, four were important agronomic traits: tuber number, tuber yield, marketable rate and specific gravity. There was hardly a case to select in which all the desirable traits were found in one combination. But there were instances in which sexual polyploid progeny based on 4x-2x crosses performed better than the locally tested TPS varieties resulting from 4x-4x crosses. Thus, there were clear indications that it is possible to improve the breeding populations which will be useful for the selection of 4x and 2x parents for the production of TPS varieties. SCA analysis for some of the agronomic traits such as tuber yield and specific gravity were relevant for selection of hybrid combinations as well as in analytic breeding. From the research, one 4x-2x combination was selected which now is in the stage of farmer trials.

SAMENVATTING

Op wereldschaal bezet aardappel van de meest belangrijke gewassen de vierde plaats. Dit tetraploide gewas werd in het laatste kwart van de zestiende eeuw in Europa geïntroduceerd. Als gevolg van veredeling en selectie is de huidige cultuuraardappel *Solanum tuberosum L. subsp. tuberosum* ontstaan. Hoewel dit gewas goed aangepast is aan de noordelijke klimaatomstandigheden met lange dag, is de prestatie van de aardappel in de subtropen en tropische regio's duidelijk minder. Met het oog op de potentie van aardappel om hoge knolopbrengsten te kunnen geven is het gewenst dit gewas beter aan te passen aan de dichter bevolkte, armere tropische en subtropische gebieden. Problemen bij het verbouwen van aardappels in de tropen en subtropen zijn dat aardappel een vegetatief vermeerderd gewas is en vatbaar is voor vele ziekten en plagen. Een specifiek probleem treedt op als knollen door virussen aangetast zijn. Zij kunnen dan niet meer als plantaardig uitgangsmateriaal gebruikt worden omdat dit tot aanzienlijke reducties in opbrengst kan leiden.

Een idee dat de laatste jaren veel aandacht gekregen heeft is de ontwikkeling van een aardappelras gebaseerd op zaailingpopulaties vanuit botanisch zaad (True Potato Seed; TPS), dat via 4x-4x kruisingen verkregen is. Theoretisch zijn er veel voordelen te bedenken om deze methode van rasontwikkeling te gebruiken:. 1. er worden veel aardappelziekten, vooral virusziekten, niet door TPS overgedragen; 2. het is buitengewoon goedkoop om een gewas dmv TPS te verbouwen en het bevat niet het risico om duur pootgoed te moeten gebruiken; 3. de boeren kunnen hun eigen uitgangszaad produceren; 4. tenslotte kunnen grote hoeveelheden knollen die anders als pootgoed gebruikt moeten worden nu als voedsel worden ingezet. Er zijn aan TPS rassen echter ook nadelen en problemen verbonden die opgelost moeten worden voordat TPS-rassen op grote schaal praktisch acceptabel kunnen zijn. Enkele van deze problemen zijn: 1. de opbrengst van zaailingen na uitplanten is relatief laag; 2. het geoogste product is niet zo uniform als het product dat via knolvermeerdering verkregen wordt; 3. een nieuwe teeltpraktijk moet ontwikkeld worden.

De eerste twee genoemde problemen kunnen voor een deel opgelost worden door het toepassen van 2n-gameten (het chromosoomgetal is dan niet gehalveerd in de gameten) bij de productie van TPS-rassen via 4x-2x kruisingen. In zulke kruisingen worden, vanwege een triploiden blok, 4x nakomelingen verwacht. Er zijn voldoende aanwijzingen dat als deze 2n-gameten door een specifieke fout in de meiose ontstaan, die genetisch "first division restitution" (FDR) tot gevolg heeft, de heterozygotie van de 2x-ouder in de gameet grotendeels intact blijft en dat daardoor de verkregen zaailingen ook genetisch meer uniform zullen zijn. Dit opent de weg tot het produceren van TPS rassen die naast dat ze erg heterozygoot (met heterosis) ook fenotypisch uniform zijn. Experimenteel onderzoek aan het gebruik van 2n-gameten heeft inderdaad aangetoond dat TPS effectief geproduceerd kan worden door 4x-2x kruisingen waarbij FDR 2n-gameten een belangrijke rol spelen.

Ondanks vele eerdere pogingen in verschillende landen om aardappel via TPS te telen werd het eerste merkbare succes in China verkregen. Omdat in China verschillende typen van agroklimatologische regio's te vinden zijn waar aardappel verbouwd wordt is daar de potentie duidelijk aanwezig aardappelrassen niet alleen via de traditionele veredelingsmethode maar ook via TPS te ontwikkelen. Daarnaast bleek het nodig te zijn de mogelijkheden te ontwikkelen om via analytische veredeling met diploiden tot zowel TPS rassen als traditionele rassen te komen. Het was van belang het plantmateriaal dat voor dit doel bij de vakgroep Plantenveredeling ontwikkeld was met de beschikbare basisinformatie erbij te gebruiken. Nadat de experimenten in de zomer van 1992 in Wageningen voorbereid waren is het plantmateriaal in 1993 en 1994 in China opgekweekt en onderzocht.

Op de eerste plaats, werd er een vergelijking gemaakt voor de frequentie van 2n-pollenproductie tussen individuele nakomelingen van diallel kruisingen tussen vijf bekende diploide klonen in verschillende klimatologische omstandigheden. Deze diploide ouderklonen die vanwege hun 2n-pollen productie in de literatuur reeds bekend waren, waren: A (USW5293-3), B (USW5295-7), C (USW5337-3), D (USW7589-2) en E (77.21010-37). Daarnaast werden enkele van deze diploide klonen (A, C en E) met drie klonen van *Solanum phureja*, die geen of bijna geen 2n-pollen produceren, gekruist. Een belangrijke conclusie was dat de frequentie 2n-pollen in geselecteerde klonen behoorlijk stabiel was. Er was variatie voor de frequentie 2n-pollen tussen de families en specifieke combinatiegeschiktheid (specific combining ability; SCA) had een grotere bijdrage tot deze variatie dan de algemene combinatiegeschiktheid (general combining ability; GCA).

Om meer inzicht te krijgen in de mogelijkheden om de frequentie 2n-pollen op te voeren werden kruisingen tussen verschillende geselecteerde klonen in de volgende vier typen combinaties gemaakt: laag x laag (lage frequentie 2n-pollen), laag x hoog, hoog x hoog en hoog x hoog-terugkruising. In totaal werden 3971 planten uit 36 combinaties op hun 2n-pollenproductie onderzocht. In de analyse voor uitsplitsing van 2n-pollenvorming, was het duidelijk dat deze eigenschap meer complex dan alleen maar monogeen overerft. Het was opvallend dat door het toepassen van herhaalde cycli van selectie op 2n-pollenvorming in de verschillende combinaties van hoog x hoog-terugkruising (10.7%) tov de laag x laag combinaties (2.4%) gemiddeld een viervoudige toename op populatieniveau verkregen kon worden.

Hoewel toename van de frequentie 2n-pollen één belangrijk doel was, was het ook van belang vast te stellen dat 2n-pollen effectief kunnen zijn in het doorgeven van nuttige eigenschappen uit de geselecteerde vaderplant naar de 4x-nakomelingschappen die via 4x-2x kruisingen verkregen zijn. Om deze mogelijkheid aan te geven werd onderzocht in welke mate resistentie tegen bruinrot, dat door de bacterie *Pseudomonas solanacearum* veroorzaakt wordt, na kruising aan de nakomelingen doorgegeven wordt. Voor dit doel werden verschillende 2x en 4x ouderklonen op bruinrot-resistentie getoetst en konden enkele resistente klonen worden geselecteerd. Uit het overervingspatroon kwam duidelijk naar voren dat bruinrot-resistentie een kwantitatieve eigenschap is. De diploide genotypen die een hoge mate van resistentie tegen bruinrot te zien gaven en ook 2n-pollen produceerden werden in verschillende ploidiecombinaties als 2x-2x en 4x-2x gekruist. Deze combinaties werden vergeleken met 4x-4x kruisingen. Het kwam duidelijk naar voren dat overdracht van resistentie tegen bruinrot het meest effectief was in 4x-2x combinaties. Dit betekent klaarblijkelijk dat het gebruik van 2n-pollen effectief is in het overdragen van een kwantitatieve eigenschap als bruinrot-resistentie naar 4x nakomelingen.

Om meer zicht te krijgen in de optimale veredeling van TPS-rassen werden drie verschillende typen van nakomelingschappen op 15 eigenschappen onderzocht. Deze nakomelingschappen waren afkomstig van 4x-2x en 4x-4x kruisingen en van 4x zelfbevruchtingen. De prestaties van deze nakomelingschappen werden zowel in de zaailing-generatie als in de knol-generatie na knolvorming geëvalueerd. Tussen de 15 onderzochte eigenschappen waren vier belangrijke landbouwkundige eigenschappen aanwezig: knolaantal, knolopbrengst, knolsortering en onder-water-gewicht (owg). Er was bijna geen individuele combinatie te vinden waarin alle gewenste eigenschappen aanwezig waren. Maar er waren regelmatig combinaties te vinden waarin 4x nakomelingschappen via 4x-2x kruisingen beter presteerden dan de lokale TPS rassen die uit 4x-4x kruisingen afkomstig waren. Er werden derhalve duidelijke aanwijzingen gevonden dat het mogelijk is om de veredelingspopulaties te verbeteren die bij de selectie van 4x en 2x ouders voor de productie van TPS rassen van cruciaal belang zijn. SCA analyse voor enkele van de landbouwkundige eigenschappen zoals knolopbrengst en owg waren relevant voor de selectie van zowel hybride combinaties als voor de analytische veredeling. Tijdens het onderzoek is één 4x-2x combinatie geselecteerd die nu in het stadium is van beproeving door de boer.

论文提要

世界上最重要的作物中，就栽培面积,区域分布和总产量而言，马铃薯名列第四位。十六世纪中下叶首先从南美洲引种到欧洲，在那里由于长期育种和选择的结果而形成了今天在世界各地普遍栽培的马铃薯*Solanum tuberosum L. subssp. tuberosum.*虽然它已经对北温带长日照的气候条件有很好的适应性，但在亚热带和热带地区的表现相对较差。马铃薯具有高产潜力，把这一作物改良适应于人口稠密,较贫穷的热带和亚热带地区是很有意义的。与温带地区不同的是在热带和亚热带地区种植马铃薯更困难，由于马铃薯是无性繁殖作物，易感染多种病虫害，特别是当块茎被病毒侵染后它们就不能用作种薯,因为那样会导致严重减产。

近年来利用实生种子种植马铃薯越来越受到重视。利用这种方式有许多优势。首先马铃薯的许多病害，特别是病毒病，不通过实生种子传播。其次，利用实生种子生产马铃薯经济上便宜可行，不存在利用昂贵种薯的风险。第三，农户能自己生产种薯。第四，可大量节省作种薯的块茎用作食物。然而，实生种子利用技术还不能令人满意，有一系列缺点和问题需要解决。例如：1.移栽实生苗产量相对较低：2.成熟期和农艺性状不如无性繁殖那样整齐：3.新技术、新方法和农艺措施需待研究开发。前两个问题可通过利用4X-2X杂交生产实生种子品种(即利用2n配子)获得某种程度上的解决。由于三倍体不实性(triploid block)。4X-2X杂交时其后代都是四倍体。业已证明，源自第一次分裂重组(FDR)的2n配子保留着亲本的杂合性而且遗传上更趋一致，显然，这为筛选高度杂合（具有杂种优势〕而且表现型一致的实生种子品种开辟了道路。本文作者的研究工作已确实证明实生种子品种能通过有性多倍化如：利用2n花粉，有效地筛选出来。

虽然过去几十年许多国家和地区都企图利用实生种子生产马铃薯、而中国首先获得了成功。由于中国有不同类型的生态气候区，培育适应于传统方式(无性繁殖品种)和实生种子利用的马铃薯新品种潜力很大。为此，很有必要探索通过“分析育种”途径培育常规品种和实生种子杂交种的可行性。本研究报告了利用Wageningen农业大学育种系(IvP)过去几十年选育的基础材料，1992年夏季在Wageningen开始准备试材、1993年和1994年在中国种植和研究的结果。

首次利用世界上著名的5个二倍体即 A(USW5293-3), B (USW5295-7), C (USW5337-3), D (USW7589-2), E (772101-37)做双列杂交，在不同环境条件下比较它们后代的2n花粉发生频率，另外还利用3个*Solanum phureja* 基因型(即IvP35、IvP48和 IvP101 它们几乎不产生2n花粉)与A,C和E基因型分别杂交，并对其后代基因型的2n花粉频率进行观察。结果发现：不同的家系间2n花粉发生频率存在变异,特殊配合力(SCA)比一般配合力(GCA)的效应大得多。

为了深入了解提高2n花粉频率的可能性，特设计如下四类不同2n花粉频率的杂交试验：1.低X低(小于5%) 2.低X高(高于5%) 3. 高 X 高4.高X高(回交)。一共观察了36个组合3971基因型的2n 花粉发生频率，分析了2n花粉发生的基因型分离方式。首次报道了这一性状遗传类型复杂，而不是单基因控制的性状。同时通过重复

轮回选择在“高X高(回交)”的群体中2n花粉平均发生频率可比原始群体(低X低)提高四倍。

当然提高2n花粉频率是一个重要的目标，同时很有必要证明2n花粉能够有效地把有用的性状从入选的父本中转移到有性多倍化后代中去。为了证明这一点，进行了马铃薯青枯病抗性转育的试验。从筛选的几份抗青枯病的二倍体和四倍体基因型杂交后代中选育出了抗性基因型。从遗传方式看，青枯病抗性是一数量性状，把具有较高抗性同时产生2n花粉的二倍体基因型与不同倍性的母本杂交，如4X-2X和2X-2X，再与常规的4X-4X杂交后代比较，结果表明，转育青枯病抗性以4X-2X杂交方式更有效。这证明利用2n配子可以有效地转育青枯病抗性这样的数量性状到四倍体后代中去。

在考虑如何评价和筛选实生种子品种的合理育种途径时对3种不同方式的杂交后代即4X-2X,4X-4X和4X自交在15个性状方面进行了观察比较。对所有这些后代在实生苗世代和无性一代的表现进行了系统评价。在调查的15个性状中有4个是重要的经济性状，即块茎数，块茎产量，商品薯率和块茎比重。当然不太可能把所有理想的性状集中到一个组合中去，但有许多4X-2X组合要比传统的4X-4X杂交组合表现优良。这清楚表明,通过选择4X和2X亲本来改良育种群体进而筛选出优良实生种子组合是完全可能的，特殊配合力分析对于杂交组合筛选和分析育种大有益处。

论文题目：利用二倍体马铃薯不减数(2n)配子通过4X-2X杂交筛选实生种子杂交种

关键词：不减数(2n)配子，二倍体马铃薯，4X-2X杂交，实生种子育种。

Curriculum Vitae

The author was born on October 29, 1963 in Hunan province of China. After his primary and secondary education for nine years, he enrolled into the Hunan Agricultural University in 1979. In 1983, he was awarded the B.Sc degree in the field of Horticultural Science. During the same year, he moved on to North China and was selected as one among four M.Sc. candidates to work on potato breeding with the two well-known potato Professors, Zhu Mingkai and late Li Jinhua in order to continue for M. Sc. degree. During that degree course, he specilized in the field of potato breeding and genetics at Northeast Agricultural University and at the Graduate School of Chinese Academy of Agricultural Sciences (CAAS). At that period, he gained experience on the aspects of 2n-gametes in potato and their use in true potato seed production, the latter of which was pioneered in China. After obtaining M.Sc. degree from the Graduate School of CAAS in early 1986, he was appointed as the project leader for the project on true potato seed (TPS) breeding and utilization. This was a collaborative project between the Institute of Vegetables and Flowers (IVF), CAAS, Beijing and International Potato Center (CIP), Lima, Peru. At the same time he was also deeply involved in the national potato programme on germplasm enhancement and breeding. Since 1992, he has mainly focused on the "Sandwich Fellowship" programme at the Institute of Vegetables and Flowers (IVF), CAAS and the Department of Plant Breeding (IvP), Wageningen Agricultural University. Since 1993, he has been serving as a senior researcher at the IVF/CAAS, Beijing, China.

Account of publications of Mr. Qu Dongyu

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