Pennisetum section *Brevivalvula* in West Africa

morphological and genetic variation in an agamic species complex

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STELLINGEN

1

Wanneer apomixis facultatief is in een complex, is het waarschijnlijk in een actieve fase van evolutie. Er zijn aanwijzingen dat deze vorm van apomixis gerelateerd is aan open of verstoorde habitats (b.v. *Rubus*, *Taraxacum*, *Hieracium*). Wanneer dit zo is, zou facultatieve apomixis gezien moeten worden als een speciale vorm van hybridizatie waarbij genotypes gevormd worden, die door selectie in een later stadium gestabiliseerd worden.

Stace, C.A., (ed.) 1975. Hybridization and
the Flora of the British Isles. Academic Press, London.

2

In zijn morfologische studie van *Pennisetum* section *Brevivalvula* classificeert Brunken *P. subangustum* als een synoniem van *P. polystachion*, de twee meerjarige soorten *P. setosum* en *P. atrichum* als ondersoorten van *P. polystachion*, en onderscheidt hij een tamelijk zeldzame hybride tussen *P. pedicellatum* en *P. polystachion* als ondersoort van *P. pedicellatum*. Dit gejongleer met taxa lijkt meer gebaseerd te zijn op de aandrang om te scoren als taxonoom dan om duidelijkheid te scheppen in de taxonomie van de sectie.

J.N. Brunken, 1979. Morphometric variation
and the classification of *Pennisetum* section
Brevivalvula (Gramineae) in tropical Africa.
Bot. J. Linn. Soc. 79: 51-64.

3

Het is te betwijfelen of het gebruik van apomictische parelgierst in de Sahel zal leiden tot hogere opbrengsten, omdat nieuwe cultivars in het algemeen hogere eisen stellen aan de bodem en de bodemvruchtbaarheid in de Sahel een groot probleem vormt.

4

Fransen mogen dan wel beweren dat hun taal betere uitdrukkingsmogelijkheden heeft dan de engelse taal, het neemt ook anderhalf keer zoveel papier in beslag.

5

In de tropen heeft de malaria profylaxis het weer verdrongen van de eerste plaats op de lijst van populaire gespreksonderwerpen.

6

Indien de invoering van de Euro gepaard zal gaan met het loslaten van de koppeling tussen de Franse frank en de munteenheid van de Franstalige Afrikaanse landen (fCFA), zal dit via economische teruggang leiden tot politieke instabiliteit in de betrokken Afrikaanse landen.

7

Commerciële bedrijven die in ontwikkelingslanden werkzaam zijn, hebben vaak een aversie tegen (non-profit) ontwikkelingssamenwerkingsprojecten, omdat deze de lokale bevolking afhankelijk zouden maken van deze projecten. "Eerlijke" handel zou daarentegen juist een onafhankelijkere positie veroorzaken, en een positieve invloed op de economie van een ontwikkelingsland uitoefenen. Helaas blijken mensen uit beide groepen te vaak alleen hun eigen broekzak belangrijk te vinden.

8

Moleculaire technieken vormen slechts een deel van de hulpmiddelen voor het oplossen van biologische vraagstukken, nooit het geheel.

9

Kleine stippen in de rivier de Niger zijn - tot spijt van velen, maar tot opluchting van de lokale bevolking - vrijwel altijd rotsen, geen nijlpaarden.

10

Er zou meer onderzoek gedaan moeten worden naar drop waar je niet misselijk van wordt zodra de zak leeg is.

11

Het milleniumprobleem is een probleem uit het jaar nul.

Stellingen behorende bij het proefschrift:

"*Pennisetum* section *Brevivalvula* in West Africa
morphological and genetic variation in an agamic species complex"

Gaby Schmelzer
Wageningen, 23 september 1998

Abstract

Section *Brevivalvula* is one of five sections in the large tropical grass genus *Pennisetum*. It belongs to the tertiary genepool of *P. glaucum* (L.) R. Br., pearl millet, and consists of six morphological species: *P. atrichum* Stapf & Hubb., *P. hordeoides* (Lam.) Steud., *P. pedicellatum* Trin., *P. polystachion* (L.) Schult., *P. setosum* (Swartz) L. Rich. and *P. subangustum* (Schum.) Stapf & Hubb. *P. setosum* and *P. atrichum* are perennials, the other species are annuals.

Four euploid (2x, 4x, 5x, and 6x with x = 9) chromosome levels are known in the section. Tetraploid cytotypes are dominant in all taxa, except *P. setosum*, which is predominantly hexaploid. Diploids and pentaploids are rare.
Some of the polyploid cytotypes of *P. pedicellatum*, *P. hordeoides*, and *P. polystachion* are at least partly facultative apomicts, while diploid *P. polystachion* and *P. subangustum* are reproducing sexually.

A morphological analysis showed that all taxa intergrade with at least one other taxon. *P. pedicellatum*, with one - or more - pedicelled spikelets in large, fluffy involucres, has differentiated from the group with a single sessile spikelet per involucrum, but intermediate plants with one almost sessile spikelet exist as well. The group with a single sessile spikelet shows a gradient from slender plants with scabrous involucral bristles on narrow inflorescences (*P. hordeoides*), to slender plants with hairy involucral bristles on narrow inflorescences (*P. subangustum*), to large plants with hairy involucral bristles on large inflorescences (*P. polystachion*), to large perennial plants with hairy involucral bristles, on large yellowish inflorescences (*P. setosum*). *P. atrichum* is grouped near *P. hordeoides*, because of its scabrous involucral bristles, but it is also perennial and rather large.

Isozyme electrophoresis of 635 plants resulted in 146 different 5-locus genotypes from combinations of 26 alleles. More than 85% of the samples of different species are connected by patterns of identical genotypes. When the proportions of 20 alleles are compared between species, ploidy levels or geographical areas, significant differences are found everywhere. The results also indicate that the samples share the same gene pool.

These results indicate that the complex is in an active state of evolution. Speciation occurs at a low rate because of sexuality in the diploids as well as facultative apomixis in part of the polyploids, which cause hybridization events among the taxa, obscuring species boundaries. Speciation can become successful when a morphotype finds a specific niche for itself. This is clearly the case for *P. pedicellatum*, which is better adapted to drier climates than the other taxa. Because of the hybridizations, a fully unambiguous description of the other species of *Pennisetum* section *Brevivalvula* is impossible.

Preface

From January 1992 till December 1996 I was assigned by DGIS (Directoraat Generaal Internationale Samenwerking) as an associate-expert in Weed Science to the project "Département de Formation en Protection des Végétaux (DFPV)" in Niamey, Niger.

In December 1992 Dr. Jean-François Renno, a geneticist at ORSTROM (Institut Français de Recherche Scientifique pour le Développement en Coopération) in Niger, who was studying wild and cultivated *Pennisetum*, asked me to assist in a collection trip of *Pennisetum* section *Brevivalvula* through southern Niger, Burkina Faso and Benin. During this trip the idea for the thesis was born, and I started my research on *Pennisetum* section *Brevivalvula* in January 1993, on a part-time basis, as the work at DFPV was there to be done as well. The research budget was assured by OSTROM and a large part of the research was effected at the Laboratory of Plants Genetics of OSTROM in Niamy (Niger).

Many people have supported me throughout the research, in Niamey, and in the Netherlands, till the long last end.

In the first place I am indebted to my promotor Prof. dr. L.J.G. van der Maesen, for the approval of the research subject and encouragement during the whole period. Dr. J.-F. Renno, my co-promotor, I am very much obliged for the initiation of the research project, and the introduction to laboratory life in general and starch gel electrophoresis in particular. Furthermore he inspired me during many discussions on *Pennisetum*, population genetics and science in the tropics.

This thesis would not have existed without the consent of the former and present directors of DFPV, Combari Abdoulaye and Sagnia Sankung, as well as the first and second "Conseiller Technique Principal" of DFPV, Jan Smit and Herman van de Voorde, to dedicate part of the office time to this work. I thank them all for their patience, their humor and for everything I learned at the project. At DFPV I thank further all the staff members and students for the pleasant working atmosphere, the discussions on crop-weed interactions in *Pennisetum*, and for all the help with the pot experiments.

At ORSTOM in Niamey especially dr. Giles Besançon and dr. Thierry Winkel are thanked for the remarks and discussions on several of the manuscripts. The competent assistance of Moussa Tidjani with the many electrophoretic analyses is highly appreciated and I thank Moussa Djibo for taking care of the plants from seed to maturity, and from there between herbarium sheets.

Dr. Hans den Nijs and Dr. Ted Mes from the University of Amsterdam I thank for their willingness with which they fitted me in their overcrowded program, for discussions on apomixis and the valuable comments on Chapter 5.

Back to Wageningen, I would like to thank Prof. dr. M.T.M. Willemse of the Experimental Plant Morphology and Cell Biology Group for introducing me to the world of embryo sacs, patiently going through all the phases of embedding and clearing methods, and explaining everything to the smallest detail.

Dr. Hans de Jong of the department of Genetics, and part of his laboratory team, took care of the chromosome counts used in Chapter 2. Furthermore, his critical reading of Chapter 5 and subtle comments were of great help.

The staff, Ph.D. students and project workers of the Plant Taxonomy Group I thank for the pleasant working atmosphere. Dr. Ronald van der Berg I am thankful for his pleasant cooperation, and his useful comments on Chapter 4.

Ties Smaling from the herbarium staff I thank especially for the excellent work he made of part of my herbarium collection and also for his enthusiasm and humor, which I could appreciate especially after my first coffee of the day. Marieke Bonhof also helped making this herbarium, which is for an entomologist quite a special thing to do. I also thank her for all the literature research she did during the time I was in Niger; they were crucial for the progress of writing the articles.

Johan van Valkenburg has a special place among my best friends, for he has been, and still is, apart from the times he spends abroad, my principal contact person between Wageningen and Niger, and later Benin. I am grateful for his help and the ease with which this help was given.

I am also grateful to Wouter and Gerda Joenje, and Wietske Jongbloed for their hospitality of always having a bed and a nice plate of food ready when I needed it. The discussions with Wouter on the ecological and evolutionary aspects of *Pennisetum* section *Brevivalvula* in West Africa I will remember for a long time.

Gerda Horsthuis-Stoorvogel I thank for painting the artistic impressions of the inflorescences of *Pennisetum* section *Brevivalvula* for the cover of this thesis. Kees Schrevel invested creative energy and probably a lot of silent sweat designing the cover of the thesis. Wil Wessel is responsible for the nice line drawings of the plants throughout the thesis.

Computers, especially lap-tops, would have been much less fun to use for writing

the thesis down, if my brother Barry would not have been there to save me from the wide variety of problems they can cause. The network at the university gave a completely different dimension to computer problems.
The lay-out of the thesis, after the writing not a negligible job, was done very nicely and patiently by Josine Donders.

And then I would like to thank everybody, who was near to me in Niger, even when I was in one of my "suboptimal" moods, caused often by the workload. I start with Anneke de Rouw, who stood at the very beginning of this thesis because it was she who introduced me to my co-promotor in Niamey. I also thank her, together with Christian Valentin and their sons Rémi and later Guy, for all the pleasant Bier en Bitterballen evenings and the more than pleasant boat trips on the river Niger.

Simone van Vught and Matthias Bartholdi I thank for their friendship and hospitality and for the many perfect and funny video evenings, joke-telling evenings and other relaxing times. Those times will come again.

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I thank Alex van Gelder, who unintentionally was the link between me and my friend François Liège. François, I thank you for your advise, every time I was in Holland and you were in Benin, to live in the present time, not in the past or future, so life can be enjoyed at its best.

Lastly, I owe this thesis really to Ine Baten and Kees Schrevel, who are like parents to me and my brother since we were kids. They fully supported my choice to study Biology, and gave me the chance to explore all the horizons I wanted.
Now see where this ended!

CONTENTS

General introduction

Pennisetum and West Africa

Pennisetum is a large and important grass genus which is distributed throughout the tropics, and which has been introduced to subtropical regions.

Pearl millet (*Pennisetum glaucum* (L.) R.Br.) is the best known species, and is of great agricultural importance in the Sahel and in parts of India, as the subsistence or even main cereal. It can still be grown on poor sandy soils, with as little as 250 mm of rainfall. A research program on *Pennisetum* has been carried out by ORSTOM (l'Institut français de Recherche scientifique pour le Développement en Coopération) in Niger since the 1980's, but collection trips have been held since 1974. Till 1991, this research was mainly focused on the genetic relationships between the wild and cultivated forms of pearl millet. Because both forms are partly sympatric and interfertile, the level of degenerated pearl millet, the hybrids, in the farmer's fields can be up to 5 to 30% (Rey-Herme, 1982), causing a substantial decline in production.

After 1991, the ORSTOM program has been extended to other species of the genus, particularly those with a West African distribution. *Pennisetum* section *Brevivalvula* and *Pennisetum purpureum* Schum. (elephant grass) neatly fit in this program. In cooperation with ICRISAT (the International Crop Research Institute for the Semi-Arid Tropics), integration was sought of information from different perspectives, such as ecology, morphology, cytogenetics, biochemistry and molecular data, so that a better understanding of the nature and origin of the observed polymorphism in these *Pennisetum* species could be reached. The results of this research are of potential use for the improvement of the agronomic performance of cultivated pearl millet, e.g. by ICRISAT.

Pennisetum section *Brevivalvula*

One of the five sections of *Pennisetum* has been studied in this thesis, section *Brevivalvula*. The section has a large distribution especially in West Africa, where the species occur between the pastoral sub-saharan zone and the rain forest zone towards the coast. Several species have been introduced to other continents. The species are defined mainly on the basis of morphological characters and life cycle, but other differences between the species are their ecology, vegetative development, inflorescence color, ploidy level, and reproduction system. They are ruderals, growing predominantly under anthropogenic conditions, on waste grounds, along road sides and as secondary weeds in and along fields. Most of them are good fodder for cattle.

Especially in India the most drought tolerant species, *P. pedicellatum*, is being cultivated as a soil stabilizer and as a temporary pasture (Bhag Mal et al., 1980; Singh & Katoch, 1980).

Aim and contents of this thesis

The main aim of this thesis is to evaluate the morphological, cytological, genetical and geographical variation that exists among West African samples of *Pennisetum* section *Brevivalvula*.

Chapter 1 summarizes what is known from the literature of the characteristics of the species in the section. A key to the different morphological species is given, as well as information about the geographical distribution, cytology, reproduction systems, resistance against pests and diseases, and uses.

In Chapter 2 the sampling strategy is described, together with the first results of the ploidy level analysis, and the distribution of those ploidy levels over the species and sampled area. A part of the distribution area was sampled and analyzed first, in order to be able to adapt the sampling strategy for other regions, if necessary. This sampling strategy consisted of the collection of as much as possible variation in populations at least 50 km apart, over three major vegetation zones. Selection of the plants was based on morphological differences, in a biased way. Sampling the largest possible morphological variation seems the best way of sampling the largest genetical variation.

Because Chapter 2 was published before Chapter 1, some information from the introduction from Chapter 2 is incomplete compared to Chapter 1.

Chapter 3 evaluates the reproduction systems present in a selection of the samples, by electrophoretic analysis of progeny arrays. This method was chosen in preference to direct observations of embryo sacs, which are appropriate to estimate the relative frequency of apomictic and sexual embryo sacs of a sample at flowering stage. Progeny tests are more appropriate for understanding the processes of evolution at population level, because only the seeds that germinate are taken into account.

Chapter 4 deals with the morphological variation found in the species from the entire sampled area of the section. This morphological variation is evaluated with principal component analysis, based on 18 characters. The results were then compared to the geographical distribution of ploidy levels and of the species. This chapter builds on the results of the ploidy levels and their geographical distribution, presented in Chapter 2.

In Chapter 5 the genotypic variation of the samples is evaluated using five enzyme systems in an electrophoretic analysis. The allelic frequencies were only calculated for the diploids, not for the polyploids, for which only the presence or

absence of the alleles was noted. Several statistics for measuring genetic diversity were used, as well as correspondence analysis, to evaluate which alleles explain the genetic variation in the section. The results of this genetic study are compared with the results of the morphological study.

After these chapters, a compilation of the most important results of this thesis are given, followed by a discussion. Directions for future research are indicated.

Terminology

Several expressions frequently used in this thesis need to be defined, in order to prevent confusion with other uses of these expressions in a different context.

In this study a genitor indicates an individual sample of a plant, with a particular but unknown genotype. Different genitors can have a same genotype, in which case they are probably clones, but different genotypes originate from different genitors.

A genotype in general is the whole of the genetical composition of an individual sample. In this study, a genotype is defined by the allelic expression at 5 loci (a 5-locus genotype), which is a restriction of the total amount of genetical variation in a plant.

A chromosomal taxon is a utilitarian expression to prevent circumscriptions like "morphological species X with cytotype A is compared to morphological species Y with cytotype B", because after many of those comparisons the outline of the comparison is lost. In most morphological species several cytotypes were found (Chapter 2 and 4). The division of these species into chromosomal taxa was done in order to detect possible morphological and genetical differences based on cytotypes.

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Chapter 1

REVIEW OF *PENNISETUM* SECTION *BREVIVALVULA* (POACEAE)[1]

G.H. Schmelzer

Summary

Section *Brevivalvula* is one of five sections in the large tropical grass genus *Pennisetum*. It belongs to the tertiary genepool of *P. glaucum* (L.) R. Br., pearl millet, and consists of six morphological taxa: *P. atrichum* Stapf & Hubb., *P. hordeoides* (Lam.) Steud., *P. pedicellatum* Trin., *P. polystachion* (L.) Schult., *P. setosum* (Swartz) L. Rich. and *P. subangustum* (Schum.) Stapf & Hubb., which together form a polyploid and agamic complex. Four euploid (x = 9) and twelve aneuploid chromosome levels have been found till now; the polyploids are apomictic, while diploid populations of *P. polystachion* and *P. subangustum* are considered sexual.

The genus *Pennisetum* and its sections

The genus *Pennisetum* (bristle grass) is one of the important genera of the tribe *Paniceae*, and is widely distributed throughout the tropics. The best known species is *Pennisetum glaucum* (L.) R. Br., pearl millet, which is the most drought tolerant major cereal cultivated. It is present mainly in subsaharan Africa and India, and can still produce with as little as 250 mm of annual rainfall. *P. purpureum* Schum., elephant grass, is an important fodder species throughout the wet tropics. Several other *Pennisetum* species are agronomically important as forage species or as weeds. Research has been focused over the years on improvement of pearl millet cultivars for grain or forage at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), India and West-Africa, on the heredity of apomixis in crosses between pearl millet and apomictic taxa (Dujardin & Hanna, 1984a,b; 1989a,b) and on the improvement and use of apomictic fodder species (Patil & Singh, 1980; Whyte, 1964).

Pennisetum has been a difficult genus to classify, and taxa have been placed formerly under a variety of genera: *Penicillaria*, *Holcus*, *Panicum*, *Setaria* and *Cenchrus*, before they settled down in *Pennisetum*. The genus is mainly characterized by its inflorescence: a false spike, with spikelets on contracted axes, or spikelets

[1] Slightly modified from Euphytica 97: 1-20 (1997)

fascicled in false spikes, always surrounded by involucres; the involucres are crowded, with slender, basally free, glabrous to plumose bristles; the spikelets are sessile or pedicellate, falling with the involucres, only persistent in the cultivated species (Watson & Dallwitz, 1992). The spikelets are lanceolate to oblong, acute to obtuse; the glumes are hyaline or membranous, often unequal, the lower one very small and sometimes absent, the upper one variable, very small to as long as the lemma, with 1-9 nerves. The valve is as long as or shorter than the spikelet, lanceolate to elliptic-oblong, acute, obtuse or truncate, frequently mucronate, rarely 3-lobed. The valvule is narrow, 2-keeled, shorter or as long as the valve, or suppressed; lodicules minute or absent. The seed is mostly oblong and dorsally compressed, obovoid or subglobose (Stapf & Hubbard, 1934).

Estimations of the number of species in the genus vary from 130 to 80 species worldwide (Nath et al., 1971; Purseglove, 1972). In tropical Africa 91 (Stapf & Hubbard, 1934) to 39 (Lebrun & Stork, 1995) species occur, but the actual number can only be determined after a revision of the genus.

Chase (1921), and later Brunken (1977) made systematic studies of pearl millet, in order to clarify the confusion around its name. Nowadays, mostly Chase (1921) is followed, who determined that *Pennisetum glaucum* (L.) R. Br. is the rightful name for pearl millet. The names *P. typhoides* (Burm.) Stapf & Hubb. and *P. americanum* (L.) Leeke are still used sometimes in publications, but are synonyms. *P. glaucum* belongs to a polymorphic species in which three subspecies have been first recognized by Brunken (1977), adapted later by Van der Zon (1992): *P. glaucum* ssp. *glaucum*, the cultivated pearl millet, *P. glaucum* ssp. *violaceum* (Lam.) A. Rich., its putative wild ancestor, and *P. glaucum* ssp. *sieberianum* (Schlecht.) Stapf & Hubb., comprising all the hybrids formed between the first two subspecies. The first two subspecies remain distinct due to pre-zygotic barriers, resulting in an advantage of self-pollination (Sarr et al., 1988; Robert et al., 1991) and post-zygotic barriers, resulting in the reduction in viability of hybrid grains (Amoukou & Marchais, 1993), geographical isolation and partly overlapping flowering periods resulting in a endogamic reproduction of the wild subspecies after the cultivated subspecies finished flowering (Renno & Winkel, 1996). The classification in three subspecies is biologically not valid, although taxonomically convenient.

Harlan (1975) divided the *Pennisetum* species into gene pools on the basis of their genetic and taxonomic relationships with the cultivated species, *P. glaucum* (comprising the three subspecies), which is placed in the primary gene pool, with $2n = 2x = 14$. The secondary gene pool includes all biological species that will cross with the primary gene pool, even when the hybrids tend to be sterile. It comprises one more species, *P. purpureum*, with $2n = 4x = 28$. The tertiary gene pool is composed of species with basic numbers $x = 5, 7, 8$, and 9. Of these species the hybrids with the primary gene pool tend to be anomalous, sterile or lethal, and gene transfer is difficult to establish.

As for the sections, most authors accept those recognized by Stapf & Hubbard (1934): *Gymnothrix*, *Pennisetum*, *Penicillaria*, *Heterostachya* and *Brevivalvula*. Only Brunken (1977) included two species of section *Penicillaria* in a different section *Pennisetum*. The differences between the sections are often not very strong and can be summarized as follows: in *Gymnothrix* the spikelets are usually solitary, rarely in clusters of 2-3; the involucres are (sub-) sessile; the bristles are scaberulous, or rarely ciliate; the anthers have glabrous tips, except *P. thunbergii* Kunth. Section *Pennisetum* has 1-4 spikelets in each involucre; the spikelets are, if clustered all, alike in shape and usually in sex, or the outer sometimes male, not keeled; the bristles are ciliate, at least the inner ones. Section *Penicillaria* is further differentiated from other *Pennisetum* species by its penicillate anthers, while section *Heterostachya* has clustered, heteromorphous spikelets, the external male laterally compressed and keeled, the central hermaphrodite. The last section *Brevivalvula* is well differentiated from the other sections by the heteromorphous valves, the lower thinly membranous, often three-lobed, the upper shorter, chartaceous, smooth and shining, truncate or very obtuse, ciliolate at the apex; the rachis has decurrent wings below the scars of the fallen involucres (Stapf & Hubbard, 1934).

In Africa section *Gymnothrix* comprises 22 species when those that were formerly classified under *Beckeropsis* are included (Stapf & Hubbard, 1934). The best known species are the very variable *P. macrourum* Trin., *P. ramosum* (Hochst.) Schweinf., and *P. hohenackeri* Steud. The section *Pennisetum* comprises five species, of which *P. villosum* (R. Br.) Fresen. (feathertop), P. setaceum (Forssk.) Chiov. (fountain grass) and *P. clandestinum* Chiov. (kikuyu grass) are most common. Section *Penicillaria* comprises seven species, but five of them are probably better classified as cultivars of *P. glaucum* (L.) R. Br. subsp. *glaucum* (pearl millet); the other species is the well-known fodder grass *P. purpureum* Schum. (napier grass, elephant grass). The section *Heterostachya* comprises two species: *P. squamulatum* Fresen. and *P. tetrastachyum* K. Schum. (syn. *P. schweinfurthii* Pilg.) and according to Lebrun & Stork (1995) section *Brevivalvula* comprises three species, *P. pedicellatum* Trin., *P. polystachion* (L.) Schult. and *P. hordeoides*, (Lam.) Steud. two of which are divided into two subspecies each: *P. pedicellatum* subsp. *pedicellatum*, *P. pedicellatum* subsp. *unispiculum* Brunken, *P. polystachion* subsp. *polystachion* and *P. polystachion* subsp. *atrichum* (Stapf & Hubbard) Brunken.

The genus *Cenchrus* is closely related to *Pennisetum*, the main difference being the flattened involucral bristles, which are fused at the base in *Cenchrus*, often forming a cup, whereas in *Pennisetum* they are filiform and not fused. This difference is sometimes marginal: *C. ciliaris* L. (syn. *P. ciliare* Link.) has been shifted between the two genera for a long time, and is obviously morphologically an intermediate form. In this species the disc is only 0.5-1.5 mm large, and only the longest bristle is flattened towards the base (Launert & Pope, 1989). Characteristics which make the species seem

more closely allied to *Pennisetum* are the antrorsely scabrous bristles, retrorse in the other *Cenchrus* species, the basic chromosome number of x = 9 and the extensive occurrence of apomixis (Pohl, 1980).

Species relationships

Several analyses has been conducted the last ten years in order to determine the degree of relationship among the different *Pennisetum* species. They can be divided into analyses that explain species relationships on the basis of qualitative and quantitative phytochemical characters in different taxa, and analyses that explain patterns on a genetical base.

Subba Rao et al. (1988), Husein et al. (1990) and Saideswara Rao et al. (1991) analyzed in total 24 different phytochemicals of the almost identical group of 12 *Pennisetum* species. The first authors performed a cluster analysis with the information obtained on 3 aspects, showing neither a strict clustering for species with a same basic chromosome type, nor for species belonging to the same gene pool, nor for a same section. *P. polystachion*, the only species of section *Brevivalvula* analyzed, was found to be 60 % dissimilar with the other clusters, basically because no protein profile was obtained, and they thus confirmed its belonging to a separate section. The analysis of four isozymes showed that the species relationships among the *Pennisetum* species, originating from all five sections, was often consistent with the available cytogenetic information. In general there was a closer affinity among species of the x = 9 basic number type and among those of the x = 7 type (primary and secondary gene pool). *P. mezianum* Leeke, the only species with a x = 8 type, showed closer relationships with the x = 9 types than with both x = 7 types. There was no particular affinity among the x = 9 species of a same section. Most of the species studied, including *P. polystachion*, showed distinct individual banding patterns. The analysis of 17 free amino acid quantities in leaf extracts revealed no clear information on their phylogenetic affinity, although the profiles in itself were highly species specific. *P. polystachion* showed the least similarity with *P. orientale* (52.9 %) and the highest with *P. squamulatum* (100%). No correlation was found with ploidy level.

The results of these phytochemical analyses show basically that the present sections of *Pennisetum* are not based on other than, rather weak, morphological similarities. The clustering of *P. polystachion* into a separate group in the first analysis is based primarily on the absence of certain leaf proteins. This is questionable because no other species of section *Brevivalvula* have been analyzed, so no generalizations can be made of the section as a whole. In general, most phytochemicals seem to be rather species specific, which make them useful as biochemical markers.

When the second group of analyses, those with a genetical base, are compared, similar conclusions are reached in part. Lagudah & Hanna (1989; 1990) studied

enzyme polymorphism of leaves, and later seed proteins and prolamines, in *Pennisetum*. They used 15 wild species and 21 pearl millet inbreds and landraces, the accessions originating from different tropical regions. The first analysis shows highly polymorphic zymograms in 4 of the 6 isozymes used. They conclude that the choice of a specific enzyme system may lead to variable deductions on phylogenetic relationships in all three gene pools. For example, the three species of section *Brevivalvula* used, *P. polystachion*, *P. pedicellatum* and *P. subangustum*, are shown to be closely related on the basis of two enzymes, but highly divergent on the basis of another. In the second analysis prolamin polymorphism was found to be much higher in wild than in cultivated pearl millet, while the prolamins found in *P. purpureum*, of the secondary gene pool, show a high similarity with this first group. Differences in prolamin composition were revealed among all species of the tertiary gene pool, caused either by the different geographical origin or sometimes by ploidy level. Compared to sections *Gymnothrix*, *Pennisetum* and *Heterostachya*, the 3 species of section *Brevivalvula* showed the highest degree of species relatedness, while within this section *P. subangustum* showed more affinity to *P. polystachion* than to *P. pedicellatum*. Similar results have been obtained by Chowdhury and Smith (1988) based on mitochondrial DNA variation. In this study *P. polystachion* and *P. pedicellatum* shared 89% of the total number of restriction fragments. It was suggested that these two species be considered as one species rather than two, which is questionable because not all species of the section have been analyzed. A more general conclusion of these analyses is that although it is difficult to determine the phylogenetic relationships in especially the tertiary gene pool, section *Brevivalvula* seems to be the only one that is fairly coherent.

Pennisetum section *Brevivalvula*

Although the section *Brevivalvula* is well differentiated from the other sections of *Pennisetum*, the number of taxa in the section is not well defined. Recent flora's (Clayton & Renvoize, 1982; Launert & Pope, 1989; Van der Zon, 1992) follow Brunken (1979b) to some extent. He distinguishes, based on a morphometric analysis of 177 single-plant collections from tropical Africa, three species of which two are subdivided into subspecies, with a total of six taxa. These taxa are: *P. pedicellatum* Trin. subsp. *pedicellatum*, *P. pedicellatum* Trin. subsp. *unispiculum* Brunken, *P. hordeoides* (Lam.) Steud., *P. polystachion* (L.) Schult. subsp. *polystachion*, *P. polystachion* (L.) Schult. subsp. *setosum* (Swartz) Brunken and *P. polystachion* (L.) Schult. subsp. *atrichum* (Stapf & Hubb.) Brunken. Other authors recognize some or all subspecies of *P. polystachion* as individual species (Stapf & Hubbard, 1934; Bor, 1960; Stanfield, 1970) and especially in West Africa *P. subangustum* (Schum.) Stapf & Hubb. is recognized as a separate species from *P. polystachion* subsp. *polystachion*

(Stapf & Hubbard, 1934; Koechlin, 1962; Stanfield, 1970; Clayton, 1972; Rose Innes, 1977). Brunken (1979b) did not find clear morphological reasons to even recognize *P. subangustum* as a subspecies of *P. polystachion* though.

Key to the species

During several fieldtrips in West-Africa (Renno et al., 1995) six taxa, covering the largest polymorphism of the section and considered as morphological species, have been recognized. These species are: *P. pedicellatum* Trin., *P. hordeoides* (Lam.) Steud., *P. polystachion* (L.) Schult., *P. subangustum* (Schum.) Stapf & Hubb., *P. atrichum* Stapf & Hubb. and *P. setosum* (Swartz) L. Rich. They are differentiated as follows, based on Stapf & Hubbard (1934):

1. Spikelets in clusters of 1-5 within the involucre, at least one of the spikelets upon a pedicel of 1-3 mm long; bristles densely woolly plumose, forming a fluffy ovate involucre of 0,5-1 cm long; spikelets 4-6 mm long; colour of involucres white, pink, red or purple ..... *P. pedicellatum*
   Spikelets solitary and sessile within the involucre 2
2. Bristles, or at least the inner ones, plumose to ciliate in the lower half 3
   Bristles glabrous, or the longer ones obscurely ciliate 5
3. Plants perennial, sparingly branched:
   Spikelets 3-5 mm long; false spike 8-10 mm broad, excluding the bristles; longest bristle 10-25 mm long, the other bristles more than twice as long as the spikelet; colour of involucres yellow, light brown or purplish ..... *P. setosum*
   Plants annual, profusely branching from the lower part; colour of involucres white, pink, red or deep purple..... 4
4. Spikelets 3-5 mm long; false spike 8-10 mm broad, excluding the bristles; longest bristle 15-25 mm long, the other bristles mostly more than twice as long as the spikelet..... *P. polystachion*
   Spikelets 2,5-3 mm long; false spike 3-7 mm broad, excluding the bristles; longest bristle 6-12 mm long, the bristles less than twice as long as the spikelet..... *P. subangustum*
5. Plants annual:
   False spike 4-6 mm broad, excluding the bristles; longest bristle 5-8 mm long, the others in 1 whorl of 6-11 and subequal to the spikelet to 1.5 times as long; spikelets 2,5-3,5 mm long; colour of involucre and spikelet red and/or white ..... *P. hordeoides*

Plants perennial:
False spike 7 mm broad, excluding the bristles; longest bristle 11-16 mm long, the others irregularly 2-whorled and up to twice as long as the spikelet; spikelets 3-4 mm long; colour of involucre and spikelet yellow .....*P. atrichum*

Geographical distribution

Pennisetum section *Brevivalvula* is widely spead in tropical Africa, with West Africa as the probable centre of diversity, because all species are present here (Stapf & Hubbard, 1934). All species except *P. atrichum* migrated (or were introduced) to India, and *P. pedicellatum*, *P. polystachion* and *P. setosum* migrated probably from there to South East Asia and Northern Australia. One species, *P. setosum*, has found its way even to the new world (Hitchcock, 1936, 1950; Luces de Fèbres, 1963). A more comprehensive recapitulation of the individual species is given below:

P. hordeoides has always been classified as a separate species. Its distribution area is West Africa (Rattray, 1960), and southwards to Zaire, Gabon and Angola (Fig. 1a). It is not mentioned to occur in East Africa (Stapf & Hubbard, 1934). The species is also present in India, in the northern humid regions (Stapf & Hubbard, 1934; Bor, 1960).

It is a slender annual species, often much branched, with small involucres. The bristles are few and bare so that the spikelet, often reddish tinged, is visible. It is a weed of cultivation, and covers often considerate areas under subhumid conditions. It is also locally abundant on disturbed sandy or gravelly sites, chiefly roadsides.

P. pedicellatum is widely distributed in West Africa (Rattray, 1960; White, 1986), except for the rain forest area in the south, but including the Cap Verde islands. The area is extended eastwards towards Ethiopia (Stapf & Hubbard, 1934), and Tanzania (Clayton & Renvoize, 1982). The species is also mentioned in Zambia (Clayton & Renvoize, 1982; Kativu & Mithen, 1988), but not in Zaire or other countries in southern Africa (Fig. 1b). It also occurs in India (Stapf & Hubbard, 1934; Bor, 1960) and has been introduced in South East Asia and western and northern Australia (Hafliger & Scholz, 1980; Webster, 1987; Lonsdale & Lane, 1994).

It is a profusely branching annual, rarely perennial species, up to 1.2 m high, with big, fluffy inflorescences. It is often dominant upon fallow land in the drier savanna, on sandy soils, and is also found around villages, on the banks of rivers, or as a weedy species on disturbed sites and road verges. It is one of the first to appear at the beginning of the rainy period.

P. pedicellatum is known as a weed in grain sorghum crops in northern Australia, and is effectively controled by hygiene, combined with minimum tillage and herbicide application. In pasture, heavy grazing prevents seed set (Groves, 1991). Maillet (1991) lists it among the most important locally abundant weeds in tropical cereals, especially

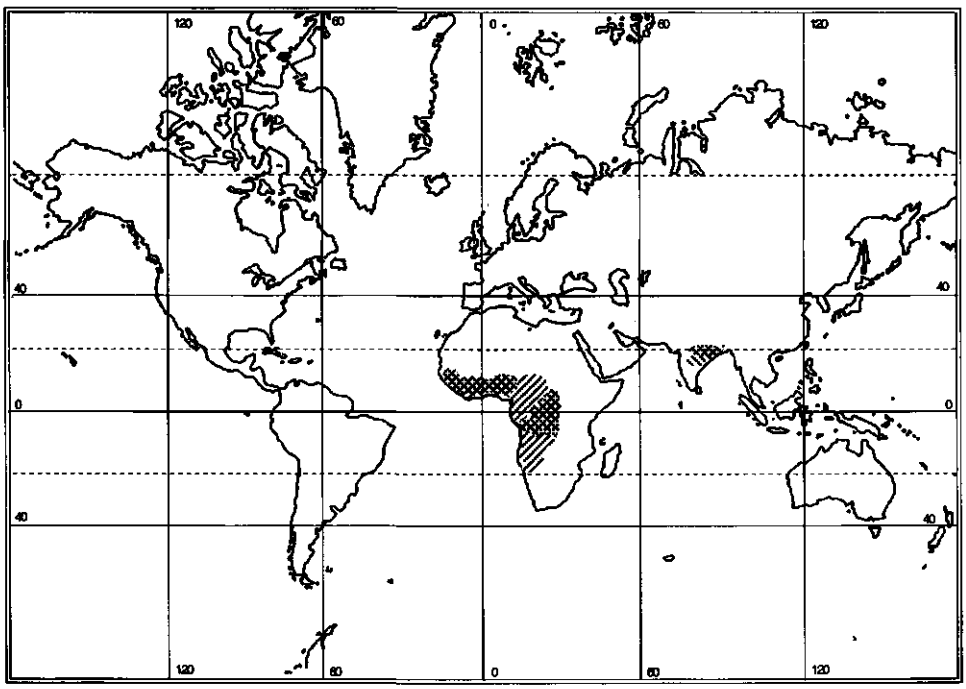

Fig.1a

P. hordeoides

P. subangustum

P. hordeoides + P. subangustum

Figure 1a: Geographical distribution of *P. hordeoides* and *P. subangustum*

in well-fertilized sorghum and pearl millet fields in Africa and Asia. Terry (1991) lists it erroneously among the perennial grasses of secondary importance in the world, while it is normally an annual. In the North of Cameroun, it is an important weed in late weeded fields, because the robust bunches are difficult either to turn over to dry out or to bury. Contact herbicides have only a limited success, because the plant can form new tillers from hidden nodes (Le Bourgeois & Merlier, 1995). *P. polystachion* is distributed in tropical Africa, including the Cap Verde Islands (White, 1986) over a larger area than *P. atrichum*, for it grows also under drier conditions (Fig 1b). Its

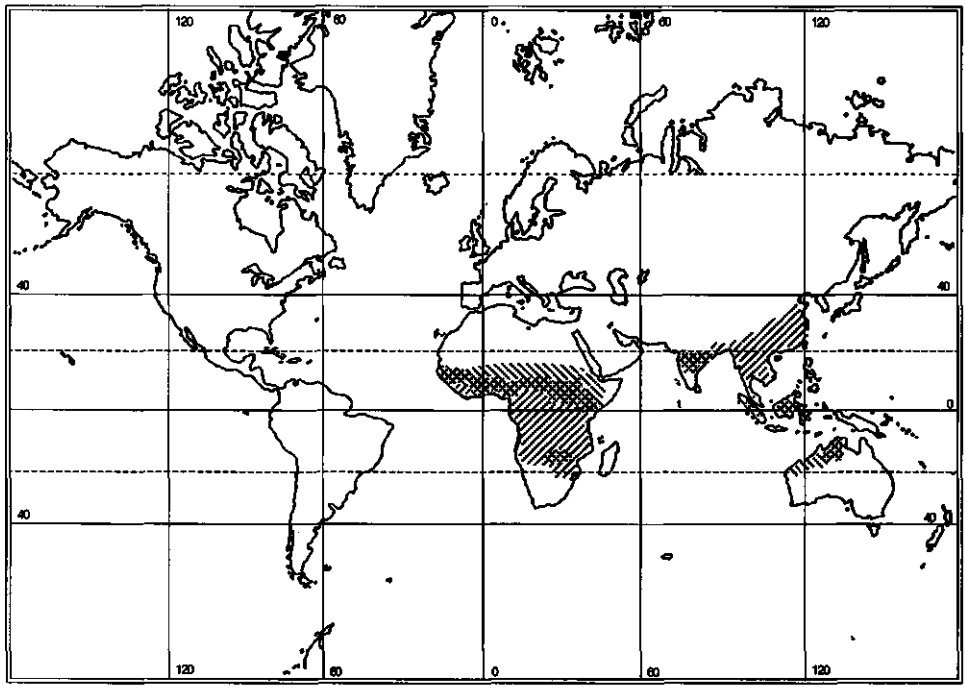

Fig.1b

Figure 1b: Geographical distribution of *P. polystachion* and *P. pedicellatum*

presence in South East Asia is uncertain despite the fact that its presence is often mentioned here, but mostly the perennial species, *P. setosum*, is meant (Hafliger & Scholz, 1980; Skerman & Riveros, 1990; Tjitrosoedirdjo, 1990). Webster (1987) mentions the presence of *P. polystachion* subsp. *polystachion* in Australia, but adds in the description that the plants are annual or perennial, while the subsp. *polystachion* is an annual (Brunken, 1979b).

It is a profusely branching annual species, up to 1.5 m high, more robust than *P. subangustum*. It grows on old farmland and waste places, and is very common,

especially as a secondary weed of disturbed roadsides, fallow land and villages. It provides good grazing during the rainy season (Rose Innes, 1977). Maillet (1991) lists it among the most important weeds in sorghum and pearl millet in Africa and Asia. Le Bourgeois & Merlier (1995) mention its presence in crops the same way as *P. pedicellatum*. Lonsdale & Lane (1994) found the involucres of *P. polystachion* being dispersed on the wheels of tourist vehicles in a national park in northern Australia, and valuated it as one of the most dangerous introduced grass weeds there. No mention was made of its lifecycle.

The name *P. polystachion* seems to be particularly prone to misspelling. The orthographic variant *P. polystachyon* is very commonly encountered, and even *P. polystachyum* occurs.

P. atrichum has a continuous distribution from Senegal in West Africa towards Kenya in East Africa and towards Zimbabwe in southern Africa (Fig. 1c). It is mentioned either as a distinct species (Stapf & Hubbard, 1934; Jackson & Wiehe, 1958; Napper, 1965; Stanfield, 1970; Clayton, 1972) or more recently, as a subspecies of *P. polystachion* (Brunken, 1979b; Clayton & Renvoize, 1982; Launert & Pope, 1989; van der Zon, 1992), which comprises annual and perennial taxa.

It is a perennial species, growing in leafy clumps till 1.8 m high, with few branches. It grows under relatively humid conditions in tree savanas, or in seasonally flooded or damp grassland, on sandy or clay soils.

P. setosum is mentioned either as a distinct species (Hitchcock, 1936, 1950; Bor, 1960; Luces de Febres, 1963), as a synonym of *P. polystachion* (Stapf & Hubbard, 1934; Koechlin, 1962; Clayton, 1972; Launert & Pope, 1989, van der Zon 1992), but then with the remark that the annual and perennial types are mixed, or as *P. polystachion* subsp. *setosum* (Brunken, 1979b). It has the largest distribution area of section *Brevivalvula* (Fig. 1c). It occurs in the whole of tropical Africa, in the subhumid to humid regions. The taxon has been introduced in India, the whole of south East Asia, the Pacific islands, and northern Australia, as well as tropical America, and there it occurs from Brazil to the south of Florida in the United States.

It is a short or long living perennial, up to 2 m high, flowering in its first year, and then resembling an annual, especially on poor soils. The species grows in clumps, with many basal leaves, and relatively few branches. It is widespread in the more humid regions, common in early stages of recolonization of abandoned cultivation and road sides. It is a noxious spreading weed in rubber plantations (Tjitrosoedirdjo, 1990), as well as oil palm plantations, orchards, vegetable and upland rice farms. Regrowth can occur from dormant buds located at the basal stem area and from the aerial nodes of the stem. As a fire disclimax, *P. setosum* invades a good deal of the mountainous land in Fiji and Thailand, and it is generally seen as a weed (Skerman & Riveros, 1990). Control by slashing does not control the plants completely, because of its easy regrowth; contact herbicides are used in plantation crops, but repeated application is

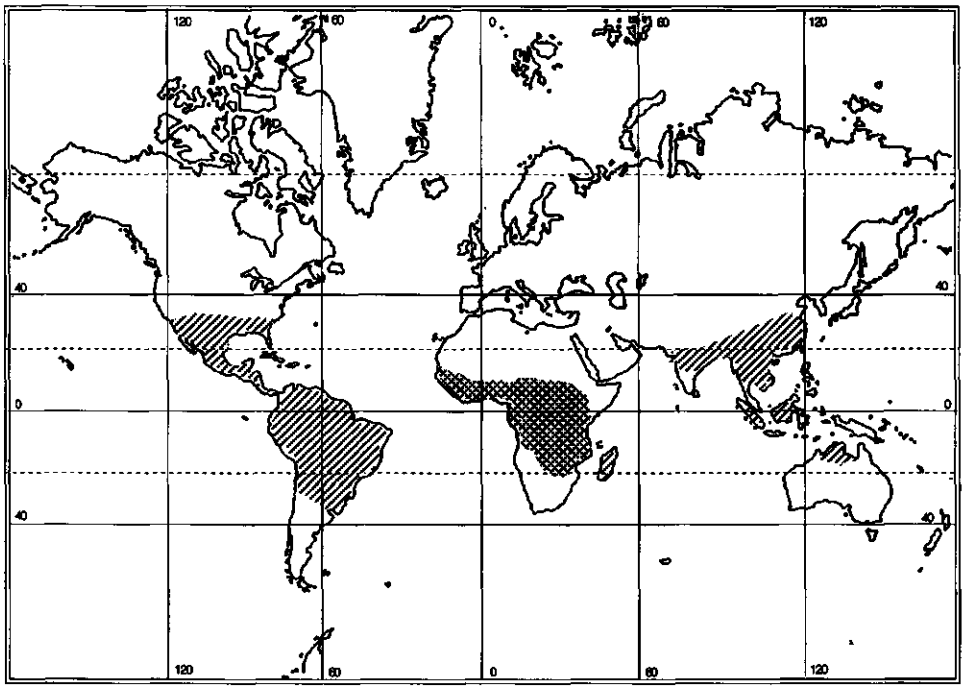

Figure 1c: Geographical distribution of *P. setosum* and *P. atrichum*

necessary (Tjitrosoedirdjo, 1990).

Of *P. subangustum* it is difficult to assess its distribution area because it is often integrated in the polymorphic species *P. polystachion*, as a synonym (Brunken, 1979b; Clayton & Renvoize, 1982; van der Zon, 1992). Before Brunken (1979b) it was classified as a separate species (Stapf & Hubbard, 1934; Chase & Niles, 1962; Koechlin, 1962; Stanfield, 1970; Clayton, 1972; Rose Innes, 1977). Herbarium material contains mostly specimens from West Africa (pers. obs. Kew, Paris, Wageningen), with few specimens originating from central Africa (Zaire, Gabon), in a disconinuous pattern (Fig. 1a). It is a common species in the savanna of the southern

half of Senegal (Stancioff et al., 1986). It has been introduced to India in the fifties,possibly with seed samples originating from Nigeria (Chatterjee & Kumar, 1964).

It is an annual species, up to 1.2 m high, with notably narrow inflorescences. It grows on fallow land and disturbed soils, with numerous other secondary weeds, under (sub)humid conditions. It is one of the dominant species in fallowed rice fields in Sierra Leone (Nyoka, 1982), in association with *P. hordeoides* (Rattray, 1960).

P. polystachion and *P. setosum* are often considered to be only one species, *P. polystachion*, which has an annual or perennial lifecycle. It is true that these species can not easily be separated in herbarium material, because often only the upper parts of the flowering culms have been collected, so that neither the branching pattern, nor the basal leaves, nor the root system can be evaluated, but this is mainly a collection problem. Another difficulty for proper identification is that the often yellowish to reddish colour of the inflorescences of *P. setosum* does not keep well in a dried state. The controversy of the lifecycles of both species has probably arisen because of their introduction into regions with different growth seasons. *P. setosum*, the perennial species, does not support long periods of drought, and will die some months after the rains have stopped. It thus behaves as an annual, but will become perennial in regions with a continuous rainy season, or a short dry season. It is not a strong species, prone to overgrazing (Skerman & Riveros, 1990) and in that case possibly does not survive many years. If allowed to grow and flower undisturbed though, it becomes perennial. *P. polystachion*, on the contrary, will die once it has flowered, even with a continuous rainy season.

Cytology of section *Brevivalvula*

The genus *Pennisetum* is cytologically very heterogeneous, chromosome numbers in the species range from 2n = 10 to 2n = 78, with basic numbers x = 5, 7, 8 and 9, while aneuploids are fairly common.

Brunken (1979a) and Jauhar (1981) cite a fairly complete list of chromosome numbers found in the taxa of *Pennisetum* section *Brevivalvula* (x = 9). A revised version of the chromosome numbers found in this section is given in Table 1. A total of four euploid and twelve aneuploid levels is found, respectively 2n = 18, 36, 45 and 54, and 2n = 24, 30, 32, 35, 35 + 1B, 42, 48, 52, 53, 56, 63 and 78. The only species with all four euploidy levels is *P. polystachion*, 2n = 18, 36, 45 and 54, and three aneuploid numbers (2n = 48, 52 and 63). *P. pedicellatum* follows with three euploid levels, 2n = 36, 45 and 54, but most aneuploids have been determined here, 9 in total (2n = 24, 30, 32, 35, 35 + 1B, 42, 48, 52, 53). *P. subangustum* shows two euploid (2n = 36 and 54) and two aneuploid (2n = 24 and 32) levels, while *P. setosum* is a predominant hexaploid species (2n = 54), with three aneuploid chromosome numbers

Table 1. Chromosome numbers of *Pennisetum* section *Brevivalvula*.

Species	2n	References
P. atrichum	36	Brunken (1979a)
P. hordeoides	18	Khosla & Mehra (1973)
	36	Renno et al. (1995)
	54	Renno et al. (1995)
P. pedicellatum	24*	Joshi et al. (1959)
	30*	Chatterji & Pillai (1970)
	32*, 35*	Carnahan & Hill (1961)
	35 + 1B*	Brunken (1979a)
	36	Brunken (1979a), Nath et al. (1971), Nath & Swaminathan (1957), Olorode (1974), Patil et al. (1964), Rangasamy (1972), Veyret (1957), Yadav et al. (1980), Renno et al. (1995)
	42*	Chatterji & Sahu (1982)
	45	Brunken (1979a), Renno et al. (1995)
	48*	Joshi et al. (1959), Chatterji & Das (1979), Chatterji & Sahu (1982)
	52*	Brunken (1979a)
	53*	Yadav et al. (1980)
	54	Brunken (1979a), Khosla & Mehra (1973), Mitra & Datta (1967), Nath et al. (1971), Nath & Swaminathan (1957), Olorode (1975) Sisodia (1970), Rangasamy (1972) Yadav et al. (1980), Renno et al. (1995)
P. polystachion	18	Renno et al. (1995)
	36	Brunken (1979a), Pantulu (1969), Renno et al. (1995)
	45	Brunken (1979a), Renno et al. (1995)
	48*	Gould & Soderstrom (1974)
	52*	Gould & Soderstrom (1974)
	54	Brunken (1979a), Gould & Soderstrom (1974), Krishnaswamy & Raman (1949), Rangasamy (1972), Renno et al. (1995), Tateoka (1965)

Table 1 continued.

Species	2n	References
	63*	Muniyama & Narayan (1975)
P. setosum	53*	Pohl & Davidse (1971)
	54	Brunken (1979a), Hrishi (1952), Renno et al. (1995)
	56*	Brunken (1979a)
	78*	Gould (1965)
P. subangustum	18	Renno et al. (1995)
	24*	Joshi et al. (1959),
	32*	Joshi et al. (1959)
	36	Krishnaswamy et al. (1954), Rangasamy (1972), Renno et al. (1995), Veyret (1957)
	54	Olorode (1975), Renno et al. (1995)

*aneuploids

found (2n = 53, 56 and 78). *P. atrichum* (2n = 36) is the only species without a variation in chromosome numbers, but this could be due to the fact that the species is underrepresented in cytological studies. Of *P. hordeoides* three euploid levels have been determined (2n = 18, 36 and 54), but no aneuploids. The diploid sample (2n = 18) of *P. hordeoides* was the only one of this level known to exist for a long time, leading to some hypotheses by Brunken (1979a) about the hybrid origin of some of the taxa in the section. Other diploids, of *P. polystachion* and *P. subangustum*, were found recently though, in the Banfora area in Burkina Faso by Renno et al. (1995), which throws a different light on the possible origins of the section.

The recurrent statement that two basic numbers, x = 8 and 9, are found in a same species (Joshi et al., 1959; Vishnuvardhan & Laksmi, 1989), is based on the assumption that the chromosome number 48 is a hexaploid with x = 8. Chatterji & Das (1979) found three ploidy levels in four biotypes of *P. pedicellatum*, 2n = 36, 48 and 54, and later Chatterji & Sahu (1982) found four different ploidy levels in five biotypes, 2n = 36, 42, 48 and 54. In this last study two biotypes had both 2n = 36 and 54, a third one showed 2n = 36 and 42. The other biotypes have either 2n = 36 or 48. The authors suppose that the ploidy level 2n = 48 is due to a drop of the basic number

from x = 9 to x = 8, called erroneously "polyploid drop", indicating that the species is in an active state of evolution. It is not less plausible though, as 2n = 48 has been found in the two species with the largest variation in ploidy numbers, *P. pedicellatum* and *P. polystachion*, that 2n = 48 is an aneuploid number, originating from the pentaploid 2n = 45, with x = 9 and its number has become fixed through apomixis, thus constituting an agamic aneuploid complex.

Most aneuploid numbers in the section were determined in chromosome counts in pollen mother cells (PMCs), not on the basis of chromosome counts in somatic cells. Renno et al. (1995) did not find any aneuploids in the section, despite the large *Brevivalvula* sample (304) which was passed through DAPI flow cytometry. The polyploid taxa of section *Brevivalvula* are highly apomictic (see next sub-chapter), and pollen viability (mostly determined as pollen stainability) is often reduced due to a high percentage of aberrant meiosis in PMC (Hrishi, 1952; Naithani & Sisodia, 1966; Nath et al., 1970; Sisodia, 1970; Brunken, 1979a; Sharma et al., 1980; Sisodia & Raut, 1980; Birari, 1981a; Vishnuvardhan & Lakshmi, 1989; Dujardin & Hanna, 1984b). The chromosome counts in PMCs will therefore show a large amount of aneuploid cells, but these aneuploid numbers are normally not reflecting somatic chromosome numbers in any progeny, partly because many pollen cells are not viable and partly because pollination in pseudogamic taxa, which is the case in section *Brevivalvula*, is only necessary for endosperm formation, not for the development of the egg cell. The chromosome numerical mosaicism observed in PMCs of some biotypes of *P. pedicellatum* does not indicate polyaneuploid series nor intra-individual aneuploidy somatically, as is suggested by Vishnuvardhan and Laksmi (1989).

The statement of Jauhar (1981) that polyploid series have been found in the following perennial, vegetatively propagated forage species: *P. hordeoides*, *P. pedicellatum*, *P. polystachion* and *P. subangustum*, among species of other sections, is incorrect. They are all annual grasses, propagating solely through seeds, while only *P. pedicellatum* is known as a forage species.

Nature of polyploidy

The *Poaceae* are characterized by the occurrence of polyploid taxa. Of the genus *Pennisetum*, nearly 76% are polyploids (Jauhar, 1981). There are basically two types of polyploids: autopolyploids, which are composed of multiple sets from within one species, and allopolyploids, which are composed of sets from different species. In autopolyploids, for instance an autotetraploid, the four chromosomes have several possibilities to pair and segregate in mitosis and meiosis, because they are homologous. Paired chromosomes, like in diploids, are called bivalents, pairing of three chromosomes are called trivalents, and pairing of four chromosomes are called quadrivalents, while one unpaired chromosome is a univalent. Bivalents and

quadrivalents are most common. With higher ploidy levels, other multivalents can occur (Griffiths et al., 1993). True autopolyploids are rare in nature because the formation of trivalents and univalents are the main causes of aneuploidy, causing various levels of sterility; expression of variability is masked by homozygoty, and they lack vigor (Jauhar, 1981). Allopolyploids are formed between related species; because the chromosomes are only partially homologous, pairing of more than two chromosomes is less common. True allopolyploids are usually only formed during hybridisation tests, in nature the parental species are rarely completely differentiated. Intermediate situations between auto- and allopolyploids - hemi-autopolyploids, auto-allopolyploids, or segmental alloploids - arise when the species are closely related, and the difference between homologous chromosomes is small. In that case multivalent formation is possible, and herewith the exchange of chromosomes of different parentage (Sybenga, 1968). It will depend on the affinity among the chromosomes whether the auto- or the allopolyploid nature will dominate in the end. Another possibility is that the autopolyploid nature of a species is not expressed, by little multivalent formation. Segmental alloploids are probably preponderant in nature.

In *Pennisetum* section *Brevivalvula*, many studies have been undertaken to understand the pairing behaviour of the chromosomes of the species. Mostly PMCs were counted at metaphase I. Multivalent configurations of *P. pedicellatum* are listed in Table 2. The tetraploids show either only bivalent formation (Rangasamy, 1972; Sharma et al., 1980), which indicates an allopolyploid origin, or also multivalent formation, with uni-, tri- and quadrivalents, the latter being the most frequent. On the basis of these multivalent formations, Patil et al. (1964) and Pantulu (1969) conclude to an autopolyploid origin, despite the high bivalent frequencies. For the higher polyploidy levels, mostly Metaphase I cells with multivalents have been observed, and Pantulu (1969) and Brunken (1979a) suppose a probable autoploid origin, while others suggest an auto-alloploid origin (Rangasamy, 1972; Sharma et al., 1980) or a segmental alloploid origin because of the relatively high number of univalents (Naithani & Sisodia, 1966; Sisodia, 1970; Sisodia & Raut, 1980). The multivalent frequency observed by Pantulu (1969) however, is very low for an autopolyploid.

Multivalent configurations of the other *Brevivalvula* species are given in Table 3. Three ploidy levels (2n = 36, 45, 54) in *P. polystachion* have been analyzed, mostly with multivalent formation, except for the three hexaploids with only bivalent formation (Hrishi, 1952; Sisodia, 1970; Rangasamy, 1972). These hexaploids belong probably to *P. setosum*, the perennial hexaploid species. Some authors tend to an autoploid nature of *P. polystachion* (Pantulu, 1969; Brunken, 1979a), while others more carefully incline to an auto-alloploid origin (Birari, 1981a; Dujardin & Hanna, 1984b). Jauhar (1981) evaluates the sample of Pantulu (1969) as segmental allotetraploid. Multivalent formation comprises uni-, tri- and quadrivalents, with mostly quadrivalents, in the tetraploids. Hexavalents are mostly present in the penta-

and hexaploids, and sometimes penta- or octovalents. In a study on *P. subangustum* (2n = 36) mostly bivalents were counted (95%), in the other cases also quadrivalents (Rangasamy, 1972). He concludes to a autopolyploid nature of the sample, but with a reduced number of quadrivalents, while Jauhar (1981) contests this on the basis of the regular bivalent formation, indicating an allopolyploid nature. The only analysis of

Table 2. Multivalent configurations in *P. pedicellatum*

2n	Multivalents							References
	I	II	III	IV	V	VI	VIII	
36		18.00						Rangasamy(1972)
		18.00						Sharma et al (1980)
		13.30		2.50				Chatterji & Das (1979)
	0.20	16.50		0.70				Nath et al. (1970)
	0.75	11.75	0.27	2.74				Brunken (1979a)*
	0.94	11.84	0.31	2.61				Brunken (1979a)
	0.05	16.50	0.05	0.70				Patil et al. (1964)
	0.75	15.25	0.45	0.85				Pantulu (1969)
45	2.80	8.80	1.30	2.30	2.30			Brunken (1979a)
	4.00	11.20	2.20	1.50	1.20			Brunken (1979a)*
48	0.33	12.50		5.70				Chatterji & Das (1979)
54	6.00	19.00		1.00		1.00		Naithani & Sisodia (1966)
	0.79	24.00	0.38	0.90		0.53		Pantulu (1969)
	4.30	19.10	0.90	0.80		0.90		Vishnuvardhan & Lakshmi (1989)
	5.74	18.58	0.13	1.17		1.00		Sisodia (1970)
	6.56	17.56	0.31	1.50		1.00		Sisodia & Raut (1980)
	1.80	5.60	1.40	1.70		0.50		Vishnuvardhan & Lakshmi (1989)
	0.18	15.60	0.40	5.10	0.10	0.13		Chatterji & Das (1979)
	2.32	13.32	0.86	3.42	0.46	1.08		Brunken (1979a)
	0.50	11.10	0.40	3.10	0.30	2.70		Brunken (1979a)*
	1.60	19.00	0.80	2.70	0.06	0.20	0.60	Chatterji & Das (1979)

* *P. pedicellatum* ssp. *unispiculum*

P. atrichum (2n = 36) shows multivalent formation, ranging from uni- to hexavalents, and the species is considered an autopolyploid, probably with reduced multivalents, by Brunken (1979a). *P. setosum* (2n = 54) shows, if the analyses by Hrishi (1952), Sisodia (1970) and Rangasamy (1972) are taken into account, predominantly bivalent

formation, and the authors conclude unanimously to an allohexaploid nature of the species. Only the sample analyzed by Brunken (1979a) is different, with multivalent formation ranging from uni- to hexavalents, with a relatively high amount of uni- and quadrivalents. His conclusion is that the species has possibly an autoploid origin.

Table 3. Multivalent configurations in the other species of section *Brevivalvula*

Species and 2n	Multivalents I	II	III	IV	V	VI	VIII	References
P. polystachion								
36	0.35	14.85	0.05	1.45				Pantulu (1969)
	0.75	11.75	0.27	2.74				Brunken (1979)
45	4.20	8.40	1.50	2.10	2.10	0.10		Brunken (1979a)
54		27.00						Hrishi (1952)
		27.00						Sisodia (1970)
		27.00						Rangasamy (1972)*
	2.04	23.30	0.08	0.74				Pantulu (1969)
	1.84	13.96	0.92	3.68	0.20	0.96		Brunken (1979a)
	9.31	19.78	0.72	0.38		0.20	0.03	Birari (1981)
	0.65	15.58	0.30	3.43	0.10	0.90	0.14	Dujardin & Hanna (1984)
P. subangustum								
36		18.00						Rangasamy (1972)**
P. atrichum								
36	1.73	5.46	1.20	3.20	0.67	0.60		Brunken (1979a
P. setosum								
54	3.20	11.00	1.20	3.26	0.50	1.60		Brunken (1979a)

* 67.4% of the cases, rest showed 1-2 quadrivalents
** 95% of the cases, rest showed 1-3 quadrivalents

Apomixis in *Pennisetum*

Apomixis, in the sense of agamospermy or asexual seed formation, is a phenomenon especially encountered in the families *Asteraceae*, *Rosaceae* and *Poaceae*, which together comprise about 10% of the angiosperm species (Nogler, 1992). In the *Poaceae* apomixis occurs as four nucleate apospory in the tribes *Paniceae* and *Andropogoneae* (Brown & Emery, 1958). In *Pennisetum*, 14 species (as well as *Cenchrus ciliaris* L.) have been found to reproduce through apomixis so far (Table 4).

All species are polyploids with x = 9, except *P. massaicum* Stapf with x = 8, most of them with several euploid or aneuploid cytotypes. Of *Cenchrus ciliaris* (Bashaw, 1962), *P. flaccidum* Griseb. (Mehra & Remanandan, 1973), *P. massaicum* (Jauhar, 1981), and *P. orientale* L.C.M. Rich. (Jauhar, 1981), sexual diploids have been found to exist, apart from apomictic polyploid cytotypes. The analyzed cytotypes of *P. frutescens* Leeke, *P. latifolium* Spreng., *P. macrourum* Trin., *P. setaceum* (Forsk.) Chiov., *P. squamulatum* Fresen. and *P. villosum* R.Br. Ex Fresen., all had an apomictic reproduction, except for a tetraploid sample of *P. flaccidum*, which was found to be sexual (Mehra & Remanandan, 1973), another tetraploid sample being apomictic (Chatterji & Timothy, 1969a). Not all authors have indicated the type of apomixis found, in which case the type of reproduction is indicated with APO (apomixis), otherwise with FAC (facultative apomixis) or OBL (obligate apomixis).

In the section *Brevivalvula*, apomixis has been found in *P. pedicellatum*, *P. polystachion*, *P. setosum*, *P. subangustum* and *P. hordeoides*. *P. subangustum* (Renno et al., 1995) is the only species in the section where sexuality of the diploids has been confirmed, in diploid P. Polystachion sexual reproduction is expected. These results are consistent with Asker & Jerling (1992) who state that agamic polyploid complexes are generally polyploid, and the related sexuals diploid. Section *Brevivalvula* is a special case, because apomixis is found in annual and perennial species, while agamic complexes are normally only found in perennials. Schmelzer and Renno (1997) have not observed a significant difference of the genetic diversity in relation to the ploidy level (diploid sexuals and polyploid apomicts) in the taxa of section *Brevivalvula* studied.

Narayan (1955) has found apomixis in *P. ramosum* (Hochst.) Schweinf., *P. clandestinum* Hochst. ex Chiov. and *P. hohenackeri* Hochst ex Steud., and Brown and Emery (1958) in *P. purpureum* Schumach., but these tendencies have not been confirmed in material from other sources, and could have been coming from atypical material.

Cenchrus ciliaris L. (synonym *Pennisetum ciliare* (L.) Link) is an intermediate species between *Cenchrus* and *Pennisetum*. It is an excellent perennial fodder grass, and has been the subject of several embryological studies. Fisher et al. (1954) and Sherwood et al. (1994) found it to be a facultative apomict, though highly aposporic, with four ploidy levels, 2n = 36, 32, 40 and 54, indicating a basic chromosome number of x = 9. Multiple embryo sacs (polyembryony) were regularly observed. Snyder et al. (1955) confirmed these findings, and also found that the species is pseudogamic. Taliaferro & Bashaw (1966) and later Sherwood et al. (1994) studied the inheritance of obligate apomixis of *C. ciliaris*, after the discovery of a biotype with a high level of sexuality (Bashaw, 1962). Extensive selfing and crossing experiments in apomictic and sexual tetraploids showed that the data fit a two locus model for tetrasomic transmission, with a dominant allele (B) in one locus for sexuality, and

Table 4. Ploidy level and relation to type of reproduction in *Pennisetum* and *Cenchrus ciliaris*

Species	Ploidy level	Type of reproduction*	References
P. dubium	polyploids	FAC	Gildenhuys & Brix (1959)
P. flaccidum	5x	APO	Mehra & Remanandan (1973)
	4x	APO	Chatterjee & Timothy (1969a)
		SEX	Mehra et al. (1968), Mehra & Remanandan (1973)
	2x	SEX	Mehra & Remanandan (1973)
P. frutescens	7x	APO	Jauhar (1981)
P. latifolium		APO	Narayan (1955)
P. macrourum	6x	OBL	Dujardin & Hanna (984b)
P. massaicum	4x	FAC	D'Cruz & Reddy (1968)
		OBL	Shantamma & Narayan (1977)
	2x	SEX	Jauhar (1981)
P. orientale	4x	OBL	Chatterjee & Timothy (1969b)
	4x	APO	Rangasamy (1972)
	polyploids	FAC	Narayan (1951), Simpson & Bashaw (1969), Jauhar (1981)
	2x	SEX	Jauhar (1981)
P. setaceum	3x	APO	Avdulov (1931), Jauhar (1981)
		OBL	Rangasamy (1972)
		FAC	Hrishi (1952)
	6x	APO	Simpson & Bashaw (1969)
P. squamulatum	6x	OBL	Dujardin & Hanna (984b)
P. villosum	5x	APO	Narayan (1951), Jauhar (1981)
		OBL	Rangasamy (1972)
Brevivalvula			
P. polystachion	6x	FAC	Birari (1981b)
		OBL	Dujardin & Hanna (1984b), Chowdhury & Smith (1988)
	4x	APO	Jauhar (1981), Renno et al. (1995)
	2x	SEX	Renno et al. (1995)
P. pedicellatum	polyploids	FAC	Chatterji & Pillai (1970)
	polyploids	OBL	Kallyane & Chatterji (1981)
	4x	APO	Renno et al. (1995)
	6x	APO	Nath et al. (1971), Jauhar (1981)
P. subangustum	4x	APO	Lubbers et al. (1994)
	2x	SEX	Renno et al. (1995)

Table 4 (continued)

Species	Ploidy level	Type of reproduction*	References
P. hordeoides	4x	APO	Renno et al. (1995)
P. setosum	polyploids	APO	Jauhar (1981)
Cenchrus ciliaris	polyploids	APO	Fisher et al. (1954), Snyder et al. (1994)
	4x	OBL	Read & Bashaw (1969), Taliaferro & Bashaw (1966)
	2x	SEX	Bashaw (1962)

* APO = apomixis
FAC = facultative apomixis
OBL = obligate apomixis
SEX = sexual reproduction

another dominant allele (A) for apospory, on another locus, and which is hypostatic to B (Sherwood et al., 1994). Sexual parents thus would have the genotypes aaaabbbb, AaaaBbbb, AAaaBbbb, AAAaBbbb, or AAAABbbb, while the aposporous parents would have the genotypes Aaaabbbb, AAaabbbb, AAAabbbb, or AAAAbbbb. Crosses between *P. glaucum* and different wild apomictic species of *Pennisetum*, especially *P. squamulatum* and *P. orientale*, in order to transfer apomixis genes into *P. glaucum*, have been subject of several breeding programs, often with success (Patil & Singh, 1964; Dujardin & Hanna, 1983a,b; 1984,a,c; 1985a,b; 1986; 1987; 1988; 1989a,b; 1990; Mohindra & Minocha, 1991; Busri & Chapman, 1992; Hanna, 1979; Hanna & Dujardin, 1982; 1986; 1990; Hanna et al., 1989, 1993; Ozias-Akins et al., 1989; 1993). Crosses between hexaploid *P. pedicellatum* or *P. polystachion* with diploid or tetraploid pearl millet resulted in partial seed development with the diploid pearl millet, so that embryo culture might be a tool for recovering these hybrids (Dujardin & Hanna, 1989). Mutagene treatments with X-rays in *P. pedicellatum* (Saran & Narain, 1981), as well as treatments with other crops and other mutagens, in order to break through the apomictic barrier, had no effect on the mode of reproduction, but changed at most the morphology. Lubbers et al. (1994) analyzed 11 apomictic and 8 sexual *Pennisetum* species and found that two molecular markers, a RAPD and a RFLP/STS, were specific for the apomicts. The RAPD marker was associated with 3 species, the RFLP/STS marker with 8 species, the last one evaluated to be more closely linked to the apomixis gene(s), neither of them though with species of section *Brevivalvula*, *P. pedicellatum*, *P. polystachion* and *P. subangustum*.

Spikelet proliferation

Agamospermy, as described above, is the common way of reproduction in *Pennisetum* section *Brevivalvula*. Another form of asexual reproduction found in this section and related with the inflorescence, is spikelet proliferation. Several terms have been used to describe the phenomenon of "the conversion of the spikelet, above the two glumes, into a leafy shoot" (Arber, 1934) in grasses; vegetative proliferations (Arber, 1934), vivipary (Brown & Emery, 1958) and bulbil formation (Nair & Pillai, 1969; Pantulu, 1969) are frequently encountered. The main characteristic of these spikelets is that they are sterile and the proliferations develop without seed formation. Gustafsson (1946), followed by Nygren (1949), prefers the term vivipary, while the propagules exist of bulbils or bulblets. They distinguish several groups of vivipary, one of which is the vegetative shoot formation in the inflorescences of grasses, especially in *Agrostis, Deschampsia, Festuca* and *Poa*. In Britain, Wycherley (1954a) makes a distinction between viviparous races and occasional vegetatively proliferating plants. In viviparous races the leafy proliferation is always formed as a hereditary characteristic, it becomes detached and serves to propagate the plant. Humidity is often necessary though for the detached plants to establish themselves. An exception is *Poa bulbosa* L. var. *vivipara* Koel. which is proliferating vegetatively under dry conditions, with the bulbils surviving because they are succulent. Spikelet proliferation in plants which are not members of the viviparous races is not hereditary and occurs in the temperate zone when the day length is decreasing, or under greenhouse conditions, when there is insufficient vernalization. In this case Wycherley (1954b) prefers the older and more accurate term proliferation, while he dismisses bulbil formation as being non satisfactory, as it is applied to plants which wear bulbils in parts other than the inflorescence. Arber (1934) prefers the term vivipary to be used to germination of undetached seeds only.

In the genus *Pennisetum*, Nair & Pillai (1969) report bulbil formation for the first time in hexaploid *P. polystachion*. The proliferations exist here of two to three well developed leaves, are surrounded by the involucre and are rootless; when planted in the soil, they failed to develop normally. They suggested that the bulbils develop by modification of the spikelets into vegetative buds. Pantulu (1969) observed vegetative structures that looked like bulbils on the inflorescences of hexaploid *P. pedicellatum* plants. They were neither observed on the tetraploid race, nor on the tetraploid or hexaploid race of *P. polystachion*. He transplanted fifty of these proliferated spikelets when they obtained their maximum size and more than 50 % of these plantlets grew into mature plants.

Spikelet proliferation has been observed by me in *P. polystachion* and *P. subangustum* originating from central Benin and in *P. setosum* originating predominantly from the south of Benin, a few coming from Côte d'Ivoire. These

observations have been made in Niger, under experimental conditions, from seeds collected from the regions mentioned (Renno et al., 1995). In section *Brevivalvula* spikelet proliferation occurs in polyploid taxa: the *P. polystachion* and *P. subangustum* samples are tetraploid, the *P. setosum* sample is hexaploid. As for the survival of these proliferations, tests in which individual proliferations were transplanted in pots always failed to produce plants, while when parts of inflorescences with the proliferations still attached were put in the soil, roots would be formed, and plants would develop normally. Sometimes these vegetative proliferations would develop tiny inflorescences, while they were still attached to the inflorescence themselves, and in which normal seed developed, which were viable, and produced plants genetically identical to the mother, as was tested through electrophorestical analysis (non published data).

As Arber (1934) states, proliferations are easiest to find on plants grown during the rainy season, which indicates a dependence on external conditions, but the fact that the proliferations occur only on plants of certain origins in the case cited above, also indicates a hereditary characteristic.

Uses of section *Brevivalvula*

Fodder quality

Most *Brevivalvula* species are browsed by cattle passing through fallow land and along roads and villages. Especially *P. pedicellatum* and *P. setosum* are promising species for improving grass land quality and have been evaluated since the fifties in several countries, especially in India and Fiji. Because of its annual life cycle, *P. pedicellatum* is only suitable for temporary pastures (Whyte, 1964; Singh & Katoch, 1980), and it serves at the same time as a soil stabilizer (Bhag Mal et al., 1980) in the drier zones of India.

P. pedicellatum can stand several cuts a year for green fodder, and is generally used as a cut-and-carry green forage at ear emergence (Whyte, 1964; Skerman & Riveros, 1990) but it can be made into silage and hay (Bartha, 1970). It grows well in mixtures (Skerman & Riveros, 1990) or in rotation cropping with fodder legumes (Whyte, 1964). As a short rotation forage crop with maize or groundnuts it yields better than traditional forage grasses, especially when fertilized (Chatterjee et al., 1974), while the roots and stubbles also increase the soil fertility.

Singh and Prasad (1980) concluded on the basis of a comparative study of 38 *P. pedicellatum* genitors, that superior genotypes for fodder yield can be obtained only if selection is focused on tiller number, leaf length, leaf number and stem girth, while Singh & Arora (1970) add time of flowering and disease resistance to this list. When comparing the green fodder yield of different *P. pedicellatum* cultivars with *Sorghum bicolor* (Singh & Arora, 1970), or with other *Pennisetum* species and crosses (Singh &

Katoch, 1980; Hanna et al., 1989) the species invariably is among the best performers. The study of Chatterjee & Kumar (1964) concerned 25 so-called *P. pedicellatum* strains, which were compared for their time of flowering, seed-set and green fodder yield. However, on the basis of the description of the inflorescences and photographs, they are more likely to be a mixture of three species, *P. pedicellatum* (19 strains), *P. setosum* (1 strain, perennial, originating from Australia), and *P. subangustum* (5 strains).

The combined morphological and cytological characteristics of different biotypes of *P. pedicellatum* have been subject to many evaluations, in order to find types with a superior character set for fodder improvement. Hexaploid races were often found to perform better than the tetraploid races, but there is a large variability in economic traits. Yadav et al. (1980) and Sharma et al. (1980) found that the hexaploids flowered later, the culms were thicker and they had more and bigger leaves than the tetraploids but there were no differences between plant height, tiller number and dry matter yield. Patil and Singh (1980) and Bhag Mal et al. (1980) concluded that hexaploids in general were taller, had more tillers, a higher sugar and protein content and a higher yield than the tetraploids. However, the clustering pattern of 36 varieties did not follow their geographic distribution strictly (Bhag Mal et al., 1980), indicating other selection pressures than geographical isolation. Other studies were focused on relationships between the morphology and ploidy levels of different biotypes of *P. pedicellatum* (Chatterji & Das, 1979; Chatterji & Sahu, 1982), and it seems that biotypes based on morphology only, can contain different chromosomal races.

P. setosum is cultivated in India, Thailand and the Fiji islands. Partridge (1975) uses the name *P. polystachion*, or mission grass, but from specific characteristics mentioned like poor tillering capacity, tussucky nature, height of 2 metres and greenness at the base of the plant during the dry season, rather indicate the perennial species *P. setosum*. The species has spread throughout the drier areas of Fiji, after its introduction in the 1920's. It does not persist under heavy grazing and after flowering the stems lignify and become inedible. It makes a useful hay though if cut before maturity, but is usually cut and fed green to cattle in India, as well as in Thailand and Fiji. In Fiji *P. polystachion* is also used in intercropping trials with forage legumes (Partridge, 1975) or other fodder grasses (Roberts, 1970). In Uganda, Eggeling (1947) describes *P. polystachion* as a perennial fodder plant, so he probably means *P. setosum* as well. It provides a good bulk of fodder for two years, but is only liked by cattle when it is young. He concludes that there are probably a number of strains, some palatable, others not.

Resistance for pests and diseases

When species are being evaluated for their fodder quality, it is equally important for them to be resistant to pests and diseases, partly so that introductions do not become hosts for these pests, and partly because this resistance might be transferable to cultivated crops.

P. polystachion and four other widespread African grasses have been evaluated as host plants for two maize stem borers, *Sesamia calamistis* Hampson and *Eldana saccharina* Walker, under laboratory conditions, after they have been recurrently reported as hosts. The survival rate of the larvae on these grasses was very low, less than 10% for *S. calamistis* and less than 5% for *E. saccarina*, compared to an artificial diet, resp. 95% and 60%, or maize, resp. 30% and 19% (Shanower et al., 1993). The survival rate of the larvae on four grasses was actually close to 0%, only on *Sorghum arundinaceum* (Desv.) Stapf some larvae survived (5-10%), probably because of the relatively large stems. *P. polystachion* can therefore be disregarded as a suitable host for these two stemborers.

Wilson & Hanna (1992) studied the disease resistance of 98 wild *Pennisetum* accessions from the first gene pool and 27 from the tertiary gene pool of *Pennisetum*. The tertiary gene pool species were evaluated for resistance to six fungi species. All the species, including several accessions of *P. pedicellatum*, *P. polystachion* and *P. subangustum*, were resistant to *Puccinia substriata* var. *indica*. All species except *P. squamulatum* were resistant to *Pyricularia grisea* as well, even the only accession of *P. pedicellatum*, despite the results of Saikai et al. (1983), who found it to be susceptible, and listed the species as a new host. Reactions to the other fungi ranged from highly resistant to susceptible, so probably considerable differences exist among the provenances for disease resistance. Hoffman (1990) mentions *P. pedicellatum* as a host for a whole range of parasitic plants, in Mali: *Striga hermonthica* (Del.) Benth., *S. aspera* (Willd.) Benth., *S. passargei* Engl., *Buchnera hispida* Buch.-Ham., *Rhamphicarpa fistulosa* (Hochst.) Benth., *Cuscuta campestris* R. Br., and *Cassytha filiformis* L. In some preliminary studies on resistance of *P. pedicellatum*, *P. polystachion* and *P. hordeoides* to *Striga hermonthica* (Ngarossal & Mahamat, 1993; Sy, 1994; Koulengar, 1995) it was shown that differences in susceptibility to *Striga* do exist among the provenances, but also between accessions of a same provenance. This is conform the fact that many *Brevivalvula* species reproduce apomictically, and many genetically different clones exist.

Conclusions

In *Pennisetum* the classification of the taxa into 5 sections is mostly a matter of morphological likeness, and not based on genetic relationships. Only the relationships among taxa in section *Brevivalvula* or section *Penicillaria* seem to be based on more than morphology alone. Six morphological taxa have been recognized in *Brevivalvula*, based on the largest polymorphism found in the section in West Africa. The section has spread over most of the tropics successfully, but confusion exists on geographical distribution patterns due to incomplete herbarium material, synonymy, and the controversy on the life cycles of *P. polystachion* and *P. setosum*. Section *Brevivalvula* is a highly polyploid and agamic complex: 4 euploid and 12 aneuploid levels have been found until now, the polyploids being apomictic (facultative or obligate), the diploid *P. polystachion* and *P. subangustum*, (probably) sexual. Spikelet proliferation, an alternative form of asexual reproduction, has been observed in tetraploid *P. polystachion* and *P. subangustum*, and hexaploid *P. setosum*. *P. pedicellatum* and *P. setosum* have been evaluated as excellent fodder species, but more research needs to be done on pest and disease resistance, as some studies have shown the susceptibility of several taxa to *Striga hermonthica*.

In order to better understand the evolutionary processes active in the section, research will have to focus on identification of clones through electrophoretical and, more precise, ADN-chloroplast analyses in order to identify apomictic and sexual taxa. Other points of interest are clone formation in relation to morphology, polyploidy, apomixis and geographical distribution, reproduction plasticity of clones for forage production and the inheritance of apomixis genes in pearl millet (*P. glaucum*). These informations will help to determine as well which taxonomic ranks have to be attributed to the taxa, for the sake of an unequivocal identification.

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Pennisetum atrichum

Chapter 2

VARIATION AND GEOGRAPHICAL DISTRIBUTION OF PLOIDY LEVELS IN *PENNISETUM* SECTION *BREVIVALVULA* (POACEAE) IN BURKINA FASO, BENIN AND SOUTHERN NIGER[1]

J.-F. Renno, G.H. Schmelzer & J.H. de Jong

Summary

Pennisetum section *Brevivalvula* is a species complex characterized by polyploidy and apomixis. Ploidy levels were assessed by DAPI-flow cytometry for 304 plants of the section, originating from Burkina Faso, Benin and southern Niger. The results were confirmed for 54 plants based on chromosome counts. The samples show four euploidy levels (with x = 9) distributed among five species: *P. hordeoides* (2n = 36, 54), *P. pedicellatum* (2n = 36, 45, 54), *P. polystachion* (2n = 18, 36, 45, 54), *P. setosum* (2n = 54), and *P. subangustum* (2n = 18, 36, 54). The geographical distribution of these ploidy levels seems related to major vegetation zones present in West Africa. Diploid populations of *P. polystachion* and *P. subangustum* were found in the Banfora area, in Burkina Faso.

Introduction

Section *Brevivalvula* Stapf & C.E. Hubbard is well differentiated in the genus *Pennisetum* and includes six taxa classified as species according to morphological criteria: *P. pedicellatum* Trin., *P. hordeoides* (Lam.) Steud., *P. polystachion* (L.) Schult., *P. subangustum* (Schum.) Stapf & Hubbard, *P. atrichum* Stapf & Hubbard and *P. setosum* (Swartz) L. Rich. This section is widely distributed in the tropics and in Africa between the subsaharan zone and the humid tropics.

Brevivalvula species occur mainly in anthropic areas (Clayton, 1972) and are differentiated by their ecology, phenology, ploidy level, and reproduction system (Gupta & Minocha, 1980; Skerman & Riveros, 1990). They are good forage species and some of them possess of an apomictic reproduction system, which could be used to produce an apomictic pearl millet (*P. glaucum* (L.) R.Br.) by experimental introgresssion of new genes (Hanna & Bashaw, 1987; Savidan & Dujardin, 1992).

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[1] Plant Systematics and Evolution 198: 89-100 (1995)

It is difficult to attribute clear taxonomic ranks to the *Brevivalvula* species. Hybridization between species could exist, but limitations of genetical exchanges occur also within each species because of apomixis and differences between ploidy levels. After a morphological study of herbarium specimen, Brunken (1979b) considered *P. polystachion*, *P. setosum*, and *P. atrichum* as one species, called *P. polystachion*, divided into three subspecies, *P. polystachion* ssp. *polystachion*, *P. polystachion* ssp. *setosum* and *P. polystachion* ssp. *atrichum*. *P. subangustum* was not classified as a taxon; *P. hordeoides* and *P. pedicellatum* were maintained as separate species. Yadav & al. (1980) revealed three different chromosomal races in *P. pedicellatum* (2n = 36, 53, 54; x = 9) in India. Lagudah & Hanna (1990) mention variable ploidy levels for *P. pedicellatum* and *P. polystachion*, with respectively 2n = 36, 54 and 2n = 36, 45, 54. Samples of *P. pedicellatum* from India (Kalyane & Chatterji, 1981) and Africa, as well as samples of *P. polystachion* (Chowdury & Smith, 1988) have an obligate apomictic reproduction. Birari (1981) however, mentions facultative apomictic reproduction for *P. polystachion* and Clayton (1972) suspects possible hybridizations between the species *P. polystachion* and *P. pedicellatum*. The analysis of prolamins in *P. pedicellatum*, *P. polystachion* and *P. subangustum* samples (Lagudah & Hanna, 1990) shows that these species form a distinct group from other sections in the genus. Based on the RFLP analysis of mitochondrial DNA, Chowdhury & Smith (1988) assess *P. pedicellatum* and *P. polystachion* to be close enough to be considered as one species. After studying the karyotypes of 57 samples from sect. *Brevivalvula*, Brunken (1979a) noticed new ploidy levels and variations of ploidy within the different species without a geographical or ecological differentiation. These variations have not been used to identify taxa.

Following these approaches, the *Brevivalvula* complex seems to consist mainly of several phylogenetically close taxa, which are not conform to the mixiologic criteria of the biological species defined by Mayr (1974): the word "species" must be taken as "morphological species".

Knowing the ploidy levels in a species complex is one of the requirements for understanding its population genetics. In sect. *Brevivalvula* we assessed the geographical variation of these ploidy levels in a small part of the species distribution area, but through distinct vegetation zones and different ecological zones such as plains, areas of relief, and coastal regions.

Material and methods

Description of the taxa

Section *Brevivalvula* is easily separated from other sections of *Pennisetum* by the heteromorphic nature of the floral bracts. The lower bracts are thinly membranaceous,

often 3-lobed, while the upper bracts are hardened, pointed, smooth and shiny. The rachis has decurrent wings below each involucre in the inflorescence (Stapf & Hubbard, 1934).

The species within this section are morphologically much more difficult to define. Stapf & Hubbard (1934), Bor (1960), Clayton (1972) and Brunken (1979b) had considerable problems to classify them satisfactorily because the diagnostic characteristics of the species are overlapping.

We have determined the different taxa by using the botanical identification covering the largest polymorphism. Six taxa here considered as "morphological species" have been recognized, using the descriptions of the authors mentioned above: *P. pedicellatum*, *P. hordeoides*, *P. polystachion*, *P. subangustum*, *P. setosum*, and *P. atrichum*.

Sampling

Section *Brevivalvula* has a geographical distribution which is too large to fully assess its polymorphism. In order to estimate the variation of ploidy levels in the section, we chose an 'ecological strategy of collection' which consisted of sampling through a maximum of biotopes with minimum displacements. Two sampling transects were made by car. One transect ran from east to west in Burkina Faso and was mostly confined to the sahelian zone. The other north-south oriented transect started in Niger, and crossed Benin passing from the semi-arid subsahelian zone to the humid coastal zone through the hills of Atakora.

Samples were collected approximately every 50 kilometers along each transect, the sampling interval being defined on the basis of the environmental or botanical characteristics encountered.

Fifty-two geographical sites were sampled in Niger, Benin and Burkina Faso. About 20 plants were collected and identified for each site and their observable polymorphism (morphological diversity) was covered as well as possible.

A specimen of each identified plant and/or an original seed lot was stored in the Laboratory of Genetics of ORSTOM in Niamey for possible future observations.

Estimation of ploidy levels by flow cytometry method

To analyze samples by flow cytometry method (FCM), 17 sites were chosen among 52, in order to cover maximal taxonomical diversity, while trying to approach the geographical pattern of the species in the studied zone (Table 1). For each seed lot sampled for a genitor in situ, a progeny was analyzed at plantlet stage by FCM. The sampling method of seeds and the choice of studied sites allow quick access to morphological variability but tend to overestimate the real polymorphism of populations. So, we limited ourselves to a comparison between variations of ploidy levels in the samples studied according to the collected geographical area.

The FCM as presented by Dolezel (1991) analyzes the relative intensity of DNA fluorescence by a stain. It allows measurements of relative or absolute quantities of DNA, and applies to biotechnology, ecology, biosystematics, and population biology (Marie & Brown, 1993).

The *Pennisetum* samples were analysed by FCM by the Plant Cytometry Services (PoBox 229, 5480 AG Schijndel, The Netherlands). The samples were prepared essentially according to De Laat & al. (1987): for each sample fresh leaf material is chopped with a razor blade in an icecold neutral buffer. The buffer solution adapted from De Laat & Blaas (1984) contains: 15 mM Hepes, 1mM EDTA, 15 mM DTT, 0.5 mM spermine, 80 mM KCl, 20 mM NaCl, 300 mM sucrose, 0.2% Triton X-100 and 2 mg/l of 4',6-diaminido-2-phenylindole DAPI. After chopping, the buffer, containing cell constituents and large tissue remnants, is passed through a nylon filter of 40 µm mesh size and sent through the flow cytometer (ICP 22 of Ortho Diagnostic Systems, B-2340 Beerse Belgium). DAPI is a stain for DNA which preferentially indicates sequences of bases rich in adenine and thymine. It is impossible to measure the absolute quantity of DNA with DAPI, but using DAPI in the case of closely related taxa allows a relative measure of ploidy level, with often a better resolution than with intercaling dyes (Ulrich et al., 1988). A whole multiple of the DNA quantity allocated to the chromosomic complement (1C) corresponds to an euploidy level. Therefore a value 2C will indicate a diploid specimen (2x), 4C a tetraploid (4x), 5C a pentaploid (5x), etc. However, the relationship between the number of chromosomes and the DNA quantity is not always strict because of possible individual variations of DNA quantity for a same number of chromosomes.

A sample of chicken red blood cells (CRBC) served as internal standard for each analyzed specimen. Thus, the DNA ratio between the sample and the standard could be measured apart from a possible derivation in relation to zero level.

The coefficient of variation of samples studied varied between 2% and 9%, with 90% between 2% and 6%.

Comparison between real and estimated ploidy levels

For a selected number of plant populations, chromosome preparations were made according to the cell spreading technique of Pijnacker & Ferwerda (1984). Chromosomes were stained in 4% Giemsa in 15 mM Sörensen buffer, pH 6.8. Before the FCM analysis, chromosomes were counted for about 10 plants, providing references to interpret the first results. Later, chromosome counting was extended to 54 plants which represents approximately 20% of the samples. These plants were carefully chosen among the taxa so that values of the variation intervals of ratios obtainned through FCM would be covered, in particular the extreme values of each interval.

Table 1. Number of plants per ploidy level, according to species *P. setosum* (E), *P. hordeoides* (H), *P. polystachion* (O), *P. pedicellatum* (P), *P. subangustum* (S), their vegetation zone (I, II, III) and their original sites

Species	Zone	Site	Latitude	Longitude	2x	4x	5x	6x
E	II	38	08°55.83' N	2°35.07' E				6
E	III	43	07°23.49' N	2°04.50' E				3
E	III	48	06°27.59' N	2°21.35' E				9
H	I	23	11°16.05' N	0°40.42' E		3		1
H	II	30	10°17.53' N	2°41.91' E		4		
H	II	37	09°27.28' N	2°37.55' E		3		
O	I	2	12°49.99' N	1°41.12' E		6		
O	I	3	12°28.44' N	1°30.07' E		3		
O	I	9	12°14.79' N	0°38.14' W		4		
O	I	13	11°57.98' N	2°22.54' W		8		
O	I	54	13°06.15' N	2°22.08' E		5		
O	II	19	10°39.04' N	4°49.65' W	3	2		
O	II	21	10°39.45' N	5°09.63' W	16			
O	II	26	10°19.64' N	1°22.80' E		4		1
O	II	27	10°18.85' N	1°41.40' E		3	2	3
O	II	29	10°03.72' N	2°29.78' E		8		1
O	II	30	10°17.53' N	2°41.91' E		6		
O	II	31	11°08.83' N	2°56.76' E		1		
O	II	37	09°27.28' N	2°37.55' E		5		3
O	II	38	08°55.83' N	2°35.07' E		4		3
O	III	43	07°23.49' N	2°04.50' E		2		1
O	III	48	06°27.59' N	2°21.35' E				11
P	I	2	12°49.99' N	1°41.12' E		12		
P	I	3	12°28.44' N	1°30.07' E		11		
P	I	9	12°14.79' N	0°38.14' W		12		
P	I	13	11°57.98' N	2°22.54' W		11	3	
P	I	23	11°16.05' N	0°40.42' E		1		3
P	I	54	13°06.15' N	2°22.08' E		6		4
P	II	19	10°39.04' N	4°49.65' W		4	1	1
P	II	26	10°19.64' N	1°22.80' E				3
P	II	27	10°18.85' N	1°41.40' E		2		1
P	II	29	10°03.72' N	2°29.78' E		8		
P	II	30	10°17.53' N	2°41.91' E		3		1
P	II	31	11°08.83' N	2°56.76' E		10		
S	I	3	12°28.44' N	1°30.07' E		4		
S	I	9	12°14.79' N	0°38.14' W		1		
S	I	23	11°16.05' N	0°40.42' E		8		
S	II	19	10°39.04' N	4°49.65' W	5			
S	II	21	10°39.45' N	5°09.63' W	4			
S	II	26	10°19.64' N	1°22.80' E		9		
S	II	27	10°18.85' N	1°41.40' E		6		
S	II	29	10°03.72' N	2°29.78' E		4		
S	II	30	10°17.53' N	2°41.91' E		6		
S	II	31	11°08.83' N	2°56.76' E		7		
S	II	38	08°55.83' N	2°35.07' E		1		6
S	III	43	07°23.49' N	2°04.50' E		11		1

Preliminary study of embryo sacs

Inflorescences at early stage were collected and fixed in formalin-acetic acid-alcohol (FAA). Ovaries were dehydrated in a xylol-tertiary butyl alcohol series and embedded in paraffin, sectioned by microtome and stained in safranin-fastgreen. The embryo sacs were studied by conventional optic microscopy.

Results

Geographical distribution of the species

We sampled all species from sect. *Brevivalvula* except for *P. atrichum* because its seeds were not mature. However, this species was observed around Ouagadougou and Bobo Dioulasso. *Brevivalvula* populations were most often encountered in anthropic sites - villages, land disposal areas, margins of cultivated fields, fallow lands - but scattered populations sometimes occurred in the savannah zone, away from villages and cultivated fields.

The species collected are distributed over large vegetation zones directly related to rainfall. Isohyets run more or less parallel to latitudes in southern Niger and in Burkina Faso with isohyet 1100 mm clearly rising above the hills close to Banfora (Morel, 1992). The highest rainfall is recorded in central Benin due to the orographic anomaly of the Atakora hills (Le Barbé & al., 1993).

Each species within sect. *Brevivalvula* has its own geographical distribution through four large vegetation zones defined by White (1986) as follows (Fig. 1 and 2):

- Zone I: undifferentiated sudanian woodland
- Zone II: sudanian woodland with abundant *Isoberlinia*
- Zone III: guineo-congolian mosaic of lowland rain forest and secondary grassland
- Zone IV: guineo-congolian rain forest (drier types).

The fourth zone has not been sampled for logistical reasons.

Relationship between real and estimated ploidy level

Four separated ratio intervals were obtained after the FCM analysis, with the CRBC peak at 1.00: diploid (2x), ratio between [0.77 - 0.88], tetraploid (4x), ratio between [1.50 - 1.80], pentaploid (5x), ratio between [2.03 - 2.16], hexaploid (6x), ratio between [2.43 - 2.98].

Among the 54 plants for which the chromosomes have been counted (Table 2) only one individual at the left extremity of the interval did not have the right ploidy level estimated by FCM (4x instead of 6x, ratio 2,41). So, the other 53 plants have an estimated

ploidy level corresponding with the counted ploidy level. Consequently, to the other 250 plants analyzed only by FCM, we attributed the putative ploidy level agreeing with the interval where a particular ratio was found.

Interspecific variation of ploidy levels

P. polystachion and *P. subangustum* are significantly different at a 5% level, with a lower proportion of hexaploids in *P. subangustum*, and the presence of pentaploids in *P. polystachion* ($\chi^2 = 8.33$, d.f. = 3). They also differ from the other species because of the presence of diploid populations.

If only levels 4x and 6x are examined, *P. pedicellatum* is not significantly different from *P. subangustum*, but it differs from *P. polystachion* at the 1% level because of a higher proportion of hexaploids in *P. polystachion* ($\chi^2 = 10.45$, d.f. = 1).

P. setosum is characterized by a unique ploidy level (6x) and *P. hordeoides* shows a majority of tetraploids (91%) with only one hexaploid.

Intraspecific variation of ploidy levels

Ploidy levels of each species are organized according to their geographical distribution, as follows:

P. pedicellatum (Fig.1), was observed only in the north of the studied area. It shows 3 euploidy levels: 4x, 5x, and 6x. The 63 plants originating from vegetation zone I are tetraploids in most cases (84%); only a few are hexaploids (11%) and pentaploids (5%). The 34 plants originating from vegetation zone II are tetraploids in most cases (79%); the frequency of hexaploids reaches 18% and only one pentaploid originating from the Banfora area was observed. The difference between the geographical patterns of ploidy levels 4x and 6x is not significant between vegetation zones I and II.

P. setosum (Fig.1) occurred scarcely in the Natitingou area, and increased in frequency in the regions of Parakou and Savé down to the coast. However, mature seeds could only be collected south of Parakou. The 18 plants analyzed were all hexaploids.

P. hordeoides (Fig.1) was seen only between the cities of Pama in Burkina Faso and Savé in Benin. All 11 plants were tetraploids except for one hexaploid.

P. polystachion (Fig. 2) was observed in the whole sampled area and shows four euploidy levels: 2x, 4x, 5x, and 6x.

The 26 plants originating from vegetation zone I are all tetraploids. The 65 plants originating from vegetation zone II have variable ploidy levels. In that zone, the 44 samples from Benin are tetraploids in most cases (70%) but hexaploids appear in the Atakora relief (25%). Ploidy level variation is very noticeable in the Natitingou area: there are three euploidy levels (4x, 5x, 6x) among 13 individuals. In Burkina Faso, around the Banfora area, 90% of the analyzed plants were diploids, the others being tetraploids. The 14 plants

Table 2. Ploidy levels (2n) obtained through chromosome counting in the species *P. setosum* (E), *P. hordeoides* (H), *P. polystachion* (O) and *P. subangustum* (S). The number of cells in which chromosomes are counted is indicated

Species	Site	Ratio	2n	Cell
H	37	1.62	4x	5
H	30	1.66	4x	5
H	30	1.70	4x	8
H	30	1.72	4x	5
H	23	2.68	6x	5
O	19	0.80	2x	6
O	21	0.84	2x	6
O	21	0.87	2x	6
O	9	1.59	4x	21
O	13	1.61	4x	2
O	13	1.63	4x	3
O	3	1.66	4x	5
O	27	1.70	4x	4
O	29	1.71	4x	5
O	19	1.73	4x	2
O	27	2.08	5x	3
O	38	2.43	6x	3
O	38	2.48	6x	12
O	27	2.50	6x	3
O	48	2.69	6x	5
O	27	2.71	6x	4
O	48	2.74	6x	4
O	48	2.76	6x	8
O	38	2.88	6x	2
E	48	2.64	6x	2
E	48	2.76	6x	5
E	48	2.79	6x	6
E	38	2.82	6x	4
E	48	2.90	6x	4
E	43	2.98	6x	2
P	30	1.50	4x	6
P	9	1.60	4x	5
P	3	1.70	4x	4
P	31	1.76	4x	5
P	31	1.80	4x	7
P	13	2.03	5x	2
P	13	2.05	5x	4
P	13	2.06	5x	8
P	19	2.16	5x	8
P	27	2.41	4x	8
P	54	2.50	6x	3
P	23	2.68	6x	8
S	19	0.77	2x	5
S	19	0.80	2x	5
S	21	0.82	2x	7
S	21	0.88	2x	6
S	43	1.62	4x	4
S	26	1.70	4x	8
S	29	1.73	4x	5
S	27	1.80	4x	6
S	38	2.47	6x	6
S	38	2.54	6x	3
S	38	2.61	6x	5
S	43	2.66	6x	7

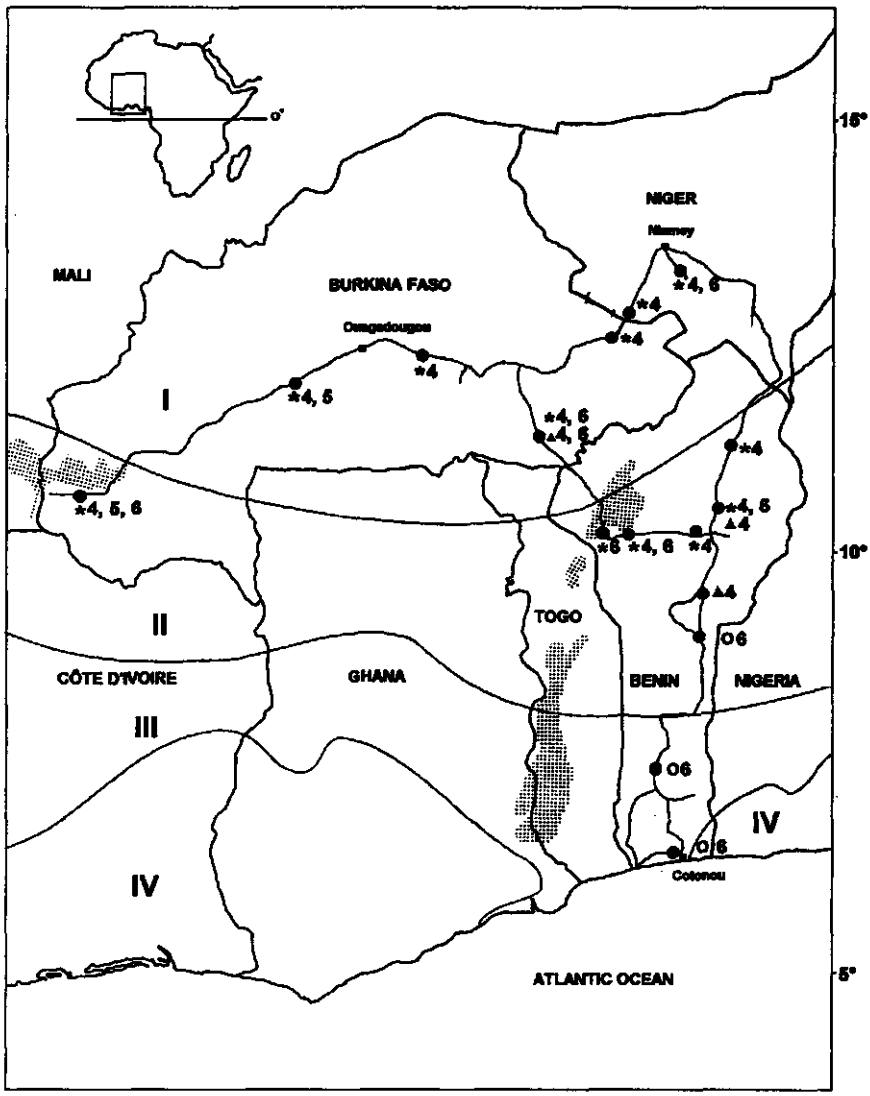

Figure 1. Geographical distribution of ploidy levels in the species *P. pedicellatum* (*), *P. hordeoides* (▲) and *P. setosum* (O). The stippled pattern represent areas of relief

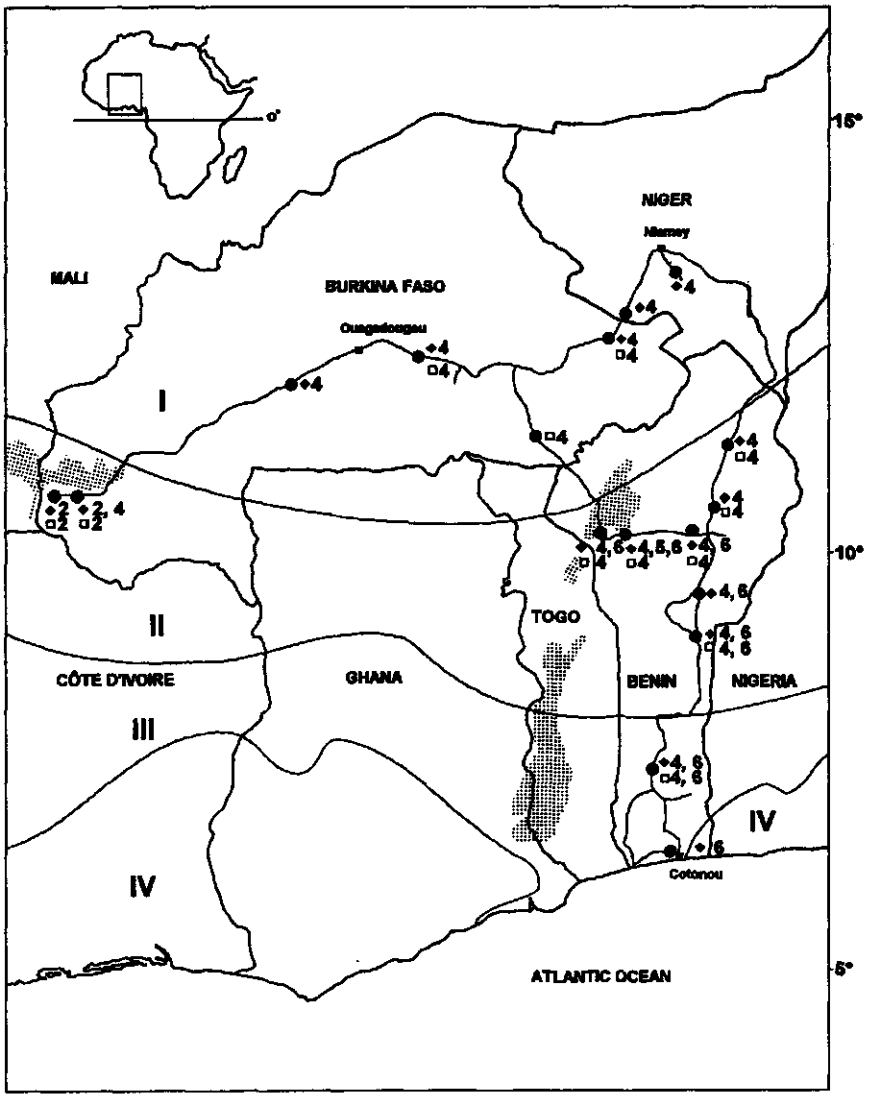

Figure 2. Geographical distribution of ploidy levels in the species *P. polystachion* (♦) and *P. subangustum* (□). The stippled pattern represent areas of relief

originating from vegetation zone III are hexaploids in most cases (86%) and the rest is tetraploid (14%).

The geographical distribution of *P. polystachion* ploidy levels cannot be tested with a χ^2 test because of empty classes in the sample. However, apparent structuring appears in relation to the vegetation zones, as follows: zone I, all plants are tetraploids, zone II, presence of hexaploids and diploids apart from the tetraploids, zone III, a majority of hexaploid plants.

P. subangustum (Fig. 2) follows the same geographical pattern as *P. polystachion* and shows three euploidy levels: 2x, 4x, and 6x. All analyzed plants (13) originating from zone I are tetraploids. The 39 samples originating from vegetation zone II in Benin are tetraploids in most cases (85%) with hexaploids (15%) occurring in the south of this area. The 9 plants from the Banfora area are all diploids. Within the vegetation zone III, tetraploids are the most encountered (92%), and only one hexaploid occurred for 12 analyzed plants.

For this species, the three vegetation zones differ because of the absence of diploids and hexaploids in zone I and of the presence of diploids only in zone II.

Preliminary study of embryo sacs

Observations of the embryo sacs of one diploid plant (2n = 18) belonging to *P. subangustum* and 4 tetraploid plants (2n = 36) belonging to *P. polystachion* (2 individuals), *P. hordeoides* (1 individual) and *P. pedicellatum* (1 individual), showed strictly sexual reproduction for the diploid specimen, while all the others had an apomictic reproduction system.

Discussion

The analysis by flow cytometry of 304 samples and chromosome counts of 54 plants have shown 4 euploidy levels among the species of section *Brevivalvula*, distributed between *P. hordeoides* (2n = 36, 54), *P. pedicellatum* (2n = 36, 45, 54), *P. polystachion* (2n = 18, 36, 45, 54), *P. setosum* (2n = 54), and *P. subangustum* (2n = 18, 36, 54). The species are different, either because they have particular ploidy levels, or they have different percentages of individuals with a given ploidy levels.

Apomicts are often polyploid and originate from hybridizations between populations of close sexual species (Bierzychudeck, 1985; Gustafsson, 1947). In general, when sexual populations are maintained in an agamic complex, they are perennials and diploids like the parental species (Asker & Jerling, 1992). The only perennial species in section *Brevivalvula* are *P. setosum* and *P. atrichum* and no diploids have been observed in these species. Previously, only one diploid had been identified in *P. hordeoides* (Khosla & Mehra, 1973). For the first time, diploid specimens have been found in *P. polystachion* and

P. subangustum and a case of strict sexual reproduction was found in *P. subangustum* (the other species has not yet been studied in this context). The fact that the species including diploid plants, with cases of strictly sexual reproduction, are annual, suggests that section *Brevivalvula* could be a special case where apomixis could have originated, at least in part, from annual populations. In dry ecological conditions, where few perennial grasses have evolved, apomixis associated with an annual life cycle would allow, through clonal multiplication of the same genotype, to increase chances of survival of this genotype by seed dispersion.

Large *Brevivalvula* populations frequently occur in anthropic conditions. Their occurrence could be related to a strong ability for anthropic dispersion, followed by an easy establishment in disturbed biotopes. The structure or level of intraspecific polymorphism could become indistinct due to the mixing of plant populations related to the migration of human populations. However, in vegetation zone I, ploidy levels within the same species are not very diversified, but in vegetation zone II, populations related to relief areas show a remarkably high number of ploidy levels. So, the greatest number of ploidy levels was observed in the Atakora and Banfora areas. Such genetical diversity could come from former or existing sexual reproduction somewhere in the distribution area of apomictic forms. However, near the Banfora relief (zone II) the presence of very localized sexual reproducing diploid populations were observed. Therefore, high variation of ploidy levels which are found in particular biotopes such as areas of relief could come from a sexual reproduction pattern. Relief areas have a greater diversity of biotopes than the plains. The ecological microvariations which they offer to plant and animal populations open large possibilities of niche differentiation, thus favoring a high level of polymorphism.

In the coastal region (zone III), where *P. setosum* appears, the proportion of hexaploids increases. Apomixis with the increase of the genome size and perennity, could be related to a higher precipitation pattern which allows the development and the preservation of such populations.

This sampling method suggests that the geographical variation of ploidy levels within each species does not depend on a random anthropic distribution, but is structured according to large phytogeographical areas. This assumption is contrary to Brunken (1979a) who did not notice any geographical structuring of variations in chromosome numbers within species from sect. *Brevivalvula*. Differential selection pressures influence the establishment and the evolution of *Brevivalvula* populations according to the variation of their ploidy level. Relationships between the diversification of adaptative strategies and ploidy levels have been emphasized (Lumaret, 1988). In some sexual polyploid species, populations tended to show different ecological preferences depending on their ploidy level. In the case of *Dactylis glomerata* from Spain, for example, diploid populations grow in woodlands and tetraploid populations in open areas (Lumaret et al., 1987a, b). Among adaptative strategies, apomixis and polyploidy often go together (De Wet, 1980). Thus,

polyploidy and the natural selection of the most efficient genetical combinations which will be fixed by apomixis, tend to produce clonal breeding lines with a high heterozygotic level (De Wet, 1971). Apomixis is a natural way of fixing and dispersing heterosis, which motivates research for its monitored introgression into cultivated plants.

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Pennisetum hordeoides

Chapter 3

GENOTYPIC VARIATION IN PROGENY OF THE AGAMIC GRASS COMPLEX *PENNISETUM* SECTION *BREVIVALVULA* IN WEST AFRICA[1]

G.H. Schmelzer & J.-F. Renno

Summary

The genotypic variation of 1180 progeny from 118 genitors belonging to five species of *Pennisetum* section *Brevivalvula* has been estimated by isozyme electrophoresis with observations of five enzymatic systems, in order to compare the type of reproduction in polyploid and diploid taxa. A total of 112 different genotypes has been found, over all species. Genotypic variation was found among all progeny of the diploid populations of *P. polystachion,* and *P. subangustum,* as a consequence of their sexual reproduction system. At the polyploid level the type of reproduction appears to be predominantly apomictic, although genotypic variation in the progeny was not rare: five tetraploid and one hexaploid *P. pedicellatum,* one pentaploid and one hexaploid *P. polystachion* and one hexaploid *P. hordeoides,* in a total of 90 genitors. Genetic relationships have been observed between the diploid sexual *P. polystachion* and *P. subangustum,* and, to a lesser extent, with the tetraploids of the same taxa as well. Tetraploid *P. polystachion* and *P. pedicellatum* share genotypes with most other chromosomal taxa.

Introduction

Pennisetum section *Brevivalvula* Stapf & C.E. Hubbard is a species complex characterized by polyploidy and apomixis. Although the section is morphologically well differentiated from the other sections of *Pennisetum*, the number of taxa is not well defined. According to Brunken (1979b) three species can be recognized, of which two are subdivided into subspecies, with a total of six taxa. According to Stapf & Hubbard (1934) and Clayton (1972) a total of six species are recognized, which do not have a complete overlap with the six taxa of Brunken. These six species are *P. atrichum* Stapf & Hubbard, *P. hordeoides* (Lam.) Steud., *P. pedicellatum* Trin.,

[1] Slightly modified from Euphytica (in press)

P. polystachion (L.) Schult., *P. setosum* (Swartz) L. Rich. and *P. subangustum* (Schum.) Stapf & Hubbard. In a review article of section *Brevivalvula*, Schmelzer (1997) cites four euploid levels (x = 9) and 12 aneuploid types.

Information on the types of reproduction systems present in section *Brevivalvula* is rather scarce and often contradictory. *P. pedicellatum* is considered a facultative apomict (Chatterji & Pillai, 1970) or an obligate apomict (Kalyane & Chatterji, 1981). *P. polystachion* is considered either a facultative apomict (Birari, 1981) or an obligate apomict (Dujardin & Hanna, 1984), while *P. subangustum* is considered apomictic by Lubbers *et al.* (1994). However, sexuality was observed in the diploid populations of *P. polystachion* and *P. subangustum* found recently in a very limited region (Renno et al., 1995; Schmelzer & Renno, 1997). Otherwise, apomixis has been found in *P. setosum* (Jauhar, 1981), and *P. hordeoides* (Renno et al., 1995).

Agamic complexes are nowadays considered to be often not strictly obligate because some sexual or partially sexual plants or strains usually exist in their natural populations (asker & Jerling, 1992). This sexuality may generate sufficient genetic variation to maintain the species under changing environments. Genetic variation provides germplasm for plant improvement. Many cultivars of perennial apomictic forage grasses, like *Paspalum dilatatum* Poir., *Cenchrus ciliaris* L., *Poa pratensis* L., and *Panicum maximum* Jacq. are obtained by breeding from the best available clones derived through residual sexuality (Bashaw & Funk, 1987). Another interesting prospect is the transfer of the gene(s) responsible for apomixis from wild apomictic plants to cultivated, sexual crops, like pearl millet, *Pennisetum glaucum* (L.) R.Br. (Hanna & Dujardin, 1982; Dujardin & Hanna, 1983), which leads to fixation of heterosis and heterozygosity. Some *Pennisetum* species are a good source of fodder. In semi-arid India *P. pedicellatum* biotypes have been evaluated for their qualitative and quantitative characteristics, and are considered superior to other fodder grasses, despite their annual lifecycle (Singh & Katoch, 1980; Skerman & Riveros, 1990). *P. setosum* is being cultivated in India, Thailand and the Fiji islands (Partridge, 1975; Skerman & Riveros, 1990), where it makes a useful hay as well, if cut before maturity. Especially *P. pedicellatum* could become very useful in the semi-arid region of West Africa, where good pasture land is becoming more and more scarce, with the increasing demand of people and cattle on land, and the irregular rainfall patterns.

In subfamily *Panicoideae*, to which *Pennisetum* belongs, aposporic embryo sacs are generally of the *Panicum* type, which are 4-nucleate, and can be distinguished from sexual embryo sacs, which are 8-nucleate (Brown & Emery, 1958). Direct obervations of embryo sacs are appropriate to estimate the relative frequency of apomictic and sexual embryo sacs of a genitor at flowering stage. However, the relative frequency of apomictic seeds which contribute to the next generation cannot be measured precisely, because all seeds do not necessarily germinate. A direct measure of the relative frequency of apomictic and sexual plants provided by progeny tests is more

appropriate for understanding the processes of evolution at the population level, than estimation by cytological techniques (Marshall & Brown, 1974). This is especially true in the *Brevivalvula* complex, where most taxa are annual, not perennial as is frequent in agamic complexes (Asker & Jerling, 1992), and each generation is obligatory to maintain the population. The aim of the present study is to evaluate, by way of genotypic variation in progeny arrays, the relative level of sexuality in polyploid and diploid taxa of section *Brevivalvula*, and to unravel the consequence of the reproduction process by which the section maintains its genetic variability.

Materials and methods

Choice of the samples

Five out of six " morphological species " as determined by Stapf & Hubbard (1934) and Clayton (1972) are used in this study: *P. setosum*, *P. hordeoides*, *P. polystachion*, *P. pedicellatum* and *P. subangustum*. The sixth species, *P. atrichum*, was not studied because no germplasm was available. The plants were grown from seeds of 118 genitors chosen from the 304 genitors collected and used in a study to determine the ploidy levels of the samples (Renno et al., 1995).
The seeds produced by each genitor are conserved separately. An (individually sampled) genitor is a plant with an unknown genotype, and in apomictic complexes different genitors can have a same genotype. The 118 samples were carefully chosen for their distribution over three vegetation zones, five species and four ploidy levels, and originate from Burkina Faso, Benin and southern Niger (Fig. 1).

Enzymatic polymorphism

Of each of the 118 genotypes a progeny of 10 seedlings was analyzed by starch gel electrophoresis. The electrophoresis and enzyme staining schedules used, followed the techniques and zymogram description of Wendel & Weeden (1989).

Five enzymatic systems were studied: phosphoglucose mutase (PGM), E.C. 5.4.2.2.; glucose-6-phosphate isomerase (GPI), E.C. 5.3.1.9.; phosphogluconate dehydrogenase (PGD), E.C. 1.1.1.44.; endopeptidase (ENP), E.C. 3.4.-.-.; and isocitrate dehydrogenase (IDH), E.C. 1.1.1.41 (42).

For each of the five loci, each different allelic combination was coded by a letter, so for each seedling the genotype at all the loci was characterized by a five letter code.

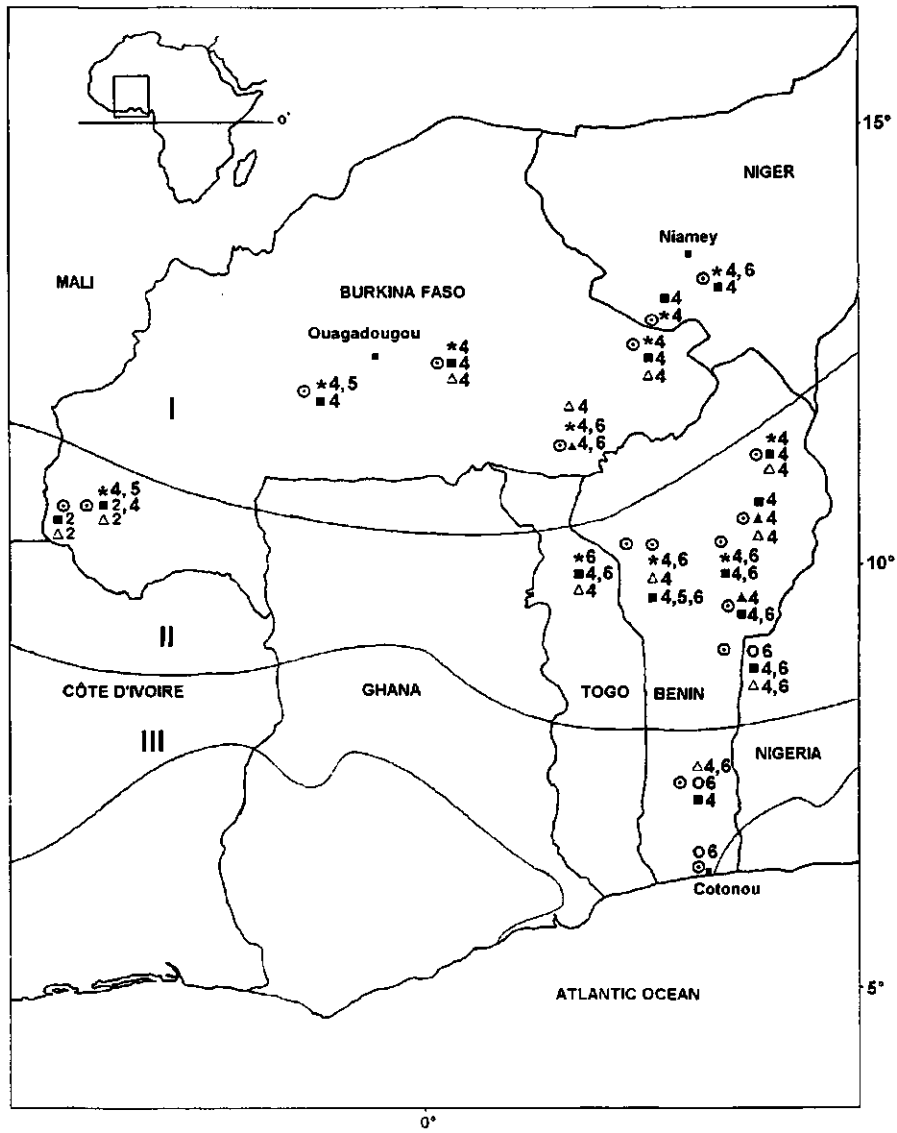

Fig. 1. Geographic distribution of the genitors over species, *P. setosum* (O), *P. hordeoides* (▲), *P. polystachion* (■), *P. pedicellatum* (*) and *P. subangustum* (▲), over the four ploidy level, diploid (2), tetraploid (4), pentaploid (5), and hexaploid (6), and over the vegetation zones, zone I = undifferentiated sudanian woodland, zone II = sudanian woodland with abundant *Isoberlinia*, zone III = guineo-congolian mosaic of rainforest and secondary grassland. The symbol ⊙ indicates the sampling site

Data analysis

Data analyses have been made on the basis of the question: Is there a significant difference among the five species, the four ploidy levels or the three vegetation zones on the chance that one or more progeny varies for at least one of the isozyme systems?

The analyses have been performed with the Generalized Linear Models procedure of the software package SAS (SAS Institute Inc., 1989), after empirical logistic transformation to achieve approximate additivity in the model (McCullagh & Nelder, 1989).

An index of heterogeneity, the index of Shannon (Greig-Smith, 1983), used commonly in plant ecology to describe the number and the evenness of the distribution of different species, is used here to describe the level of polymorphism of progeny observed in this study. The formula used is:

$$H' = - \Sigma n_i/N \times \log n_i/N$$

n_i= number of individuals with the same genotype, varying between 1 and 10
N = total number of individuals (10 progeny)
with H' varying between 0 and ∞ in general, but between 0 and 1 in this study because N=10.

Results

Enzymatic polymorphism

In all, zymograms of five enzyme systems on 1180 individual plants (118 genitors x 10 progeny) were obtained. For each of these enzymatic systems one putative locus could be interpreted. Only the position of the bands was noted, not the intensity, so it was possible to identify the presence or absence of alleles, but not to calculate allelic frequencies, resulting in a decreased polymorphism.

The putative loci used to characterize the genetic variation of the genotypes are polymorphic with a total of 24 alleles and 31 allelic combinations (Fig. 2):

- *Enp*: four monomeric isozymes corresponded to the allelic variations at one locus, eight distinct genotypes were observed in the sample.
- *Pgd*: four homodimeric isozymes corresponded to the allelic variations at one locus, three distinct genotypes were observed in the sample.
- *Pgm*: five monomeric isozymes corresponded to the allelic variations at one locus, eight distinct genotypes were observed in the sample.

- *Idh*: six homodimeric isozymes corresponded to the allelic variations at one locus, five distinct genotypes were observed in the sample.
- *Pgi*: five homodimeric isozymes corresponded to the allelic variations at one locus, seven distinct genotypes were observed in the sample.

Based on the five letter codes from the five isozyme systems, 112 genotypes were distinguished among the 1180 progeny analyzed.

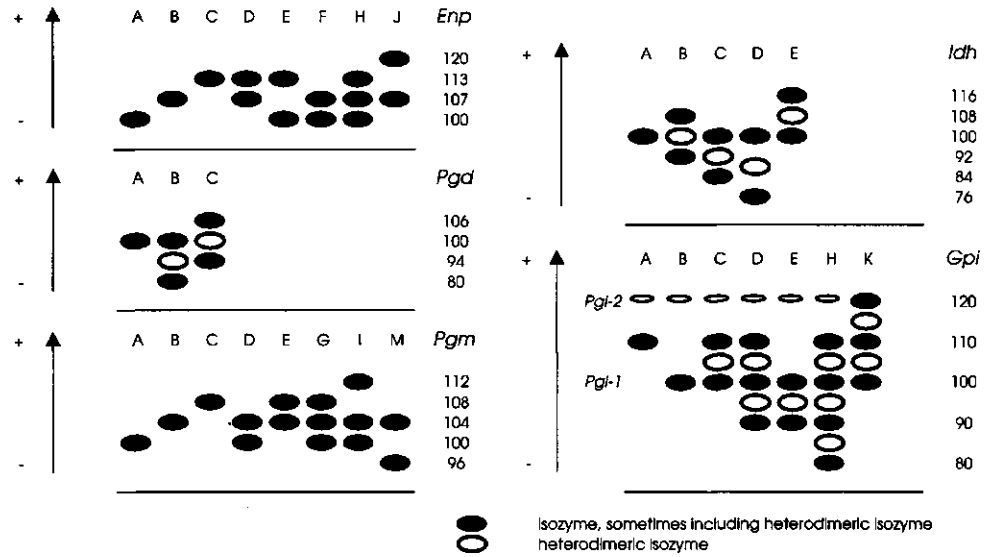

Fig. 2. Zymograms of the 5 enzyme systems, *End*, *Pgd*, *Pgm*, *Idh* and *Gpi*, with 24 allelles. The numbers indicate the names of the alleles, while the capital letters above the patterns are the names of the allelic patterns

Genotypic variation

Data are analyzed to verify whether there is a significant difference among the five species, the four ploidy levels or the three vegetation zones on the chance that one or more progeny varies for at least one of the isozyme systems, using the results shown in Table 1. There is a striking contrast between the diploid and the polyploid taxa: 19 diploid genitors (100%), and only nine polyploid genitors (9%) showed genotypic variation in their progeny (Table 2 and 3). This variation has been detected in the following polyploid taxa: five tetraploid and one hexaploid *P. pedicellatum* genitor (20,7%), one pentaploid and one hexaploid *P. polystachion* genitor (6,7%), and one

hexaploid *P. hordeoides* genitor (10%). Apart from the obvious difference between diploids and polyploids, the analysis of variance does neither show any significant difference for the vegetation zones nor for the species.

No variable progeny have been found in the tetraploid taxa of *P. polystachion*, *P. subangustum* or *P. hordeoides*, and in the hexaploid taxa of *P. setosum*, the only perennial in the sample, or of *P. subangustum* (90 genitors in total). This means that if these plants are facultative apomicts, the level of genotypic variation which cannot be detected for the five loci, with 10 progeny analyzed per locus, is less than 2%.

Table 1. Distribution of genitors with variable progeny, according to vegetation zones, species, and their ploidy level

		Ploidy level							
		2x		4x		5x		6x	
Zone[1]	Species[2]	Gen.[3]	Var.[4]	Gen.	Var.	Gen.	Var.	Gen.	Var.
I	E								
	H			3	0				
	O			6	0				
	P			11	3	3	0	5	0
	S			4	0				
II	E							3	0
	H			6	0			1	1
	O	11	11	11	0	2	1	9	1
	P			5	2	1	0	4	1
	S	8	8	8	0			6	0
III	E							7	0
	H								
	O			1	0				
	P								
	S			2	0			1	0
Total		19	19	57	5	6	1	36	3
%			100		9		16		8

[1] zone I = undifferentiated sudanian woodland, zone II = sudanian woodland with abundant *Isoberlinia*, zone III = guineo-congolian mosaic of rainforest and secondary grassland
[2] *P. setosum* (E), *P. hordeoides* (H), *P. polystachion* (O), *P. pedicellatum* (P) and *P. subangustum* (S)
[3] Number of genitors analyzed
[4] Number of genitors with variable progeny

Table 2. Polyploid genitors with variable progeny, their heterogeneity index (H'), and genotypes shared among the chromosomal taxa. The capital letters indicate the letter code given to a particular zymogram

Ploidy level	Genitor [1]	H'	Enzyme system *Enp*	*Pgd*	*Pgm*	*Idh*	*Gpi*	Nb. of progeny	Genotype shared with [1]
4x	P649	0.14	D	A	B	A	B	9	E6x,P4x,O4x
			F	A	B	A	B	1	
	P651	0.14	D	A	E	A	B	9	P4x
			D	A	B	A	B	1	E6x,P4x,O4x
	P756	0.14	D	A	G	A	C	9	O4x,H6x,P6x
			D	A	G	A	B	1	S4x,P5x
	P885	0.22	D	A	E	A	C	8	P5x
			D	A	E	A	A	2	
	P1111	0.14	D	A	D	A	E	9	
			D	A	A	A	B	1	
5x	O1042	0.14	D	A	G	A	E	9	O5x
			D	C	G	C	C	1	
6x	H956	0.35	D	A	G	A	C	7	O4x,P4x,P6x
			D	A	G	B	E	1	P6x
			D	A	G	A	A	2	
	O1017	0.14	B	A	D	C	C	9	
			C	A	D	C	C	1	
	P1024	0.14	D	A	G	C	C	9	P4x,P6x,O6x
			D	A	G	C	E	1	

[1] *P. setosum* (E), *P. hordeoides* (H), *P. polystachion* (O), *P. pedicellatum* (P) and *P. subangustum* (S). The codes in the genitor column indicate the specific sampling numbers of the genitors

Among the species and ploidy levels for which we have the most complete data set, thus only the tetra- and hexaploids of all species except *P. setosum* (E), and excluding the vegetation zones, the analysis of variance shows a significant difference for the factor ploidy level, and the interaction between species and ploidy level (P = 0.0555 resp. 0.0592). These results are caused principally by the only hexaploid genitor of *P. hordeoides*, and which has variable progeny, resulting in 100% variation. No significant difference was found between *P. pedicellatum*, the only species with variable progeny in tetraploids, and the other species.

Table 3. The heterogeneity index (H') of the 19 diploid genitors, and the loci for which they vary

Genitor [1]	Varying locus	Nb. of progeny with a same genotype	H'
O922	*Pgm, Gpi*	1+1+8	0.28
X870	*Gpi*	4+6	0.29
O871	*Idh, Gpi*	1+2+7	0.35
X917	*Gpi*	1+3+6	0.39
X874	*Enp, Gpi*	1+4+5	0.41
O872	*Enp, Pgm, Gpi*	1+1+4+4	0.52
X906	*Pgm, Gpi*	1+2+2+5	0.53
O913	*Pgm, Gpi*	1+2+3+4	0.56
X918	*Pgm, Gpi*	1+2+3+4	0.56
X880	*Enp, Pgm, Gpi*	1+1+1+2+5	0.59
O909	*Pgm, Idh, Gpi*	1+1+1+3+4	0.62
O912	*Pgm, Gpi*	1+1+2+2+4	0.64
O920	*Pgm, Gpi*	1+1+2+2+4	0.64
O873	*Pgm, Idh, Gpi*	1+1+2+3+3	0.66
O919	*Pgm, Gpi*	1+1+2+3+3	0.66
X868	*Enp, Gpi*	1+1+1+1+2+4	0.70
O911	*Pgm, Idh, Gpi*	1+1+1+2+2+3	0.73
O875	*Pgm, Idh, Gpi*	1+1+1+1+2+2+2	0.82
X907	*Pgd, Pgm, Gpi*	1+1+1+1+1+1+1+1+2	0.94

[1] see Table 2 for explications of the symbols

Heterogeneity of the variants

The results of Shannon's measure (Fig. 3 and Table 2 and 3), which includes all samples, show a relatively low level of heterogeneity in the progeny of the polyploids when compared to the diploids. An intermediate zone exists (H' = 0.2-0.3), indicating that some polyploid progeny cannot be distinguished by their genotypic heterogeneity from the diploid sexuals. The hexaploid *P. hordeoides* is at the H' = 0.3 range, indicating that it is a facultative apomict with a relatively high level of variability, and confirming its separate status compared to the other polyploids. Most polyploids, 90 genitors, have H' = 0.

Shared genotypes among all chromosomal taxa

Of the 112 genotypes distinguished, 21 are shared among two or more taxa and/or ploidy levels, from here on called the chromosomal taxa (Fig. 4). Although the results are preliminary, especially in the strength of their relations, some tendencies appear.

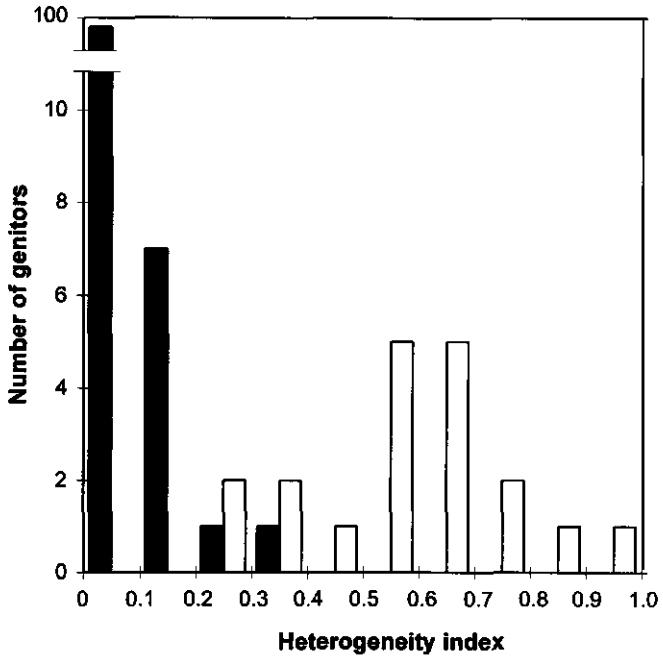

Fig. 3. Heterogeneity index of Shannon (H') of the polyploid and diploid genitors. The filled bars represent polyploids and the open bars represent diploids

Thus, the diploid taxa of *P. polystachion* (O2) and *P. subangustum* (S2) share 20% of their allelic combinations, and 2-3 % of their allelic combinations are only shared with the tetraploids of the same taxa (O4 and S4). These two tetraploid taxa do not share allelic combinations. Tetraploid *P. polystachion* (O4) and *P. pedicellatum* (P4) each share allelic combinations with seven other chromosomal taxa. Most tetraploid and hexaploid taxa are connected, while tetraploid *P. hordeoides* (H4) and pentaploid *P. polystachion* (O5) do not share any of their allelic combinations observed with other chromosomal taxa.

Discussion

The genotypic variation in the progeny can be explained by two types of fertilization:
1. " incomplete sexuality ", where only one of the gametes is reduced; and
2. " complete sexuality ", where both gametes are reduced.

Results presented here have to be interpreted only by comparison of the taxa, not in the absolute sense, because not all genetic variability has been detected. This is partly due to the restricted number of enzymes analyzed, but this is not the only reason: fertilization between reduced or non-reduced embryo sacs with reduced or non-reduced pollen probably exist and cannot always be differentiated. When, for instance, a reduced tetraploid embryo sac is fertilized by a reduced hexaploid pollen with the same alleles, this is not detected in the zymograms, as we only interpret the presence or absence of alleles, not the quantitative changes. The newly formed embryo has changed ploidy level however, and has become a pentaploid. The possible change of ploidy level, a consequence of incomplete sexuality, has not been detected because the frequency of alleles is not determined. Further studies are needed to clarify this aspect, such as measuring DNA content in variable progeny.

Both diploid populations of *P. polystachion* and *P. subangustum* always have genetically variable progeny, consistent with their sexuality. At least 9% genotypic variation is observed in the polyploids, most pronounced in tetraploid *P. pedicellatum*, but it was also detected, at a low level, in hexaploid *P. pedicellatum*, in hexaploid *P. hordeoides*, and in pentaploid and hexaploid *P. polystachion*. No variable progeny was found in polyploid *P. subangustum* and *P. setosum*. No significant differences were found among the three vegetation zones, although no variable progeny was found at all in the most humid zone III, and most of the genitors with variable progeny were found in zone II, the zone where also most variation in ploidy levels has been detected (Renno et al., 1995).

The production of unreduced gametes in diploids resulting in tetraploids is relatively common in *Andropogonae* (Savidan, 1982). One would expect more tetraploid facultative apomicts than penta- or hexaploids because in these ploidy levels the meiosis is more often disturbed. This is not confirmed in this study where tetraploids, pentaploids and hexaploids have 9%, 16% and 8% of variable progeny respectively, so the penta- and hexaploids have a comparable or higher number of facultative apomicts as the tetraploids. In addition, the only hexaploid *P. hordeoides* in the sample had a relatively high heterogeneity index, indicating a higher level of genotypic variability than the other polyploid genitors with variable progeny. It was in this species that a diploid plant has been identified first (Khosla & Mehra, 1973), which could indicate, despite the low number of analyzed plants, that *P. hordeoides* is a taxon playing an important role in the evolution of genetic variability.

Probably other genetic relations than those indicated here (Fig. 4) exist. Diploid populations of *P. polystachion* and *P. subangustum* are genetically linked either because they are phylogenetically close and/or because their diploidy level implies a restriction of genotypic combinations. Moreover, they are also linked with the tetraploids of the same taxa. The diploid species are found in a very restricted area in the Banfora area in West Burkina Faso, but could be the origin of the other taxa which have a larger colonizing ability. The tetraploid *P. polystachion* and *P. pedicellatum* are the chromosomal taxa which are widely distributed throughout the sampled regions and which share part of their allelic combinations with seven other chromosomal taxa, indicating extensive gene exchange within the whole group.

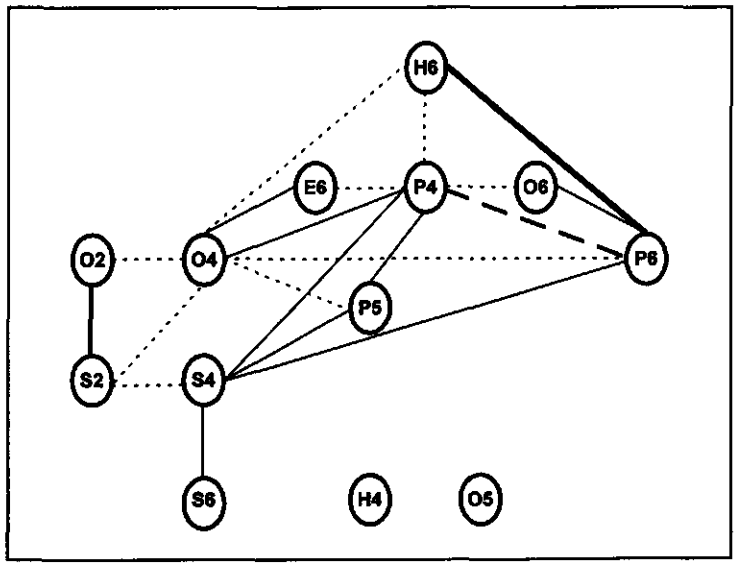

Fig. 4. Diagram of the chromosomal taxa with percentage of genotypic combinations shared: 1-5% (- - - - -), 6-10% (——), 11-15% (- - - -), 16-20 %(——), 21-25%, (——)

According to the hypothesis developed by Brunken (1979a), from a study of the cytotaxonomy of the section *Brevivalvula*, genetic exchange occurs regularly among most chromosomal taxa. Hybridizations might occur at a much higher rate than he suggests, however. A possible mechanism to produce genotypic variation in the *Brevivalvula* complex through diploid-polyploid-haploid cycles, is first described in the *Botriochloa-Dichanthium* complex with diploid and tetraploid taxa (De Wet, 1971). Sexuality is the source of genetic variation in the *Brevivalvula* section and the

tetraploid populations are the principal bridge for gene flow between diploids and the other ploidy levels. There are other grass complexes like *Calamagrostis* (Greene, 1984) and *Botriochloa-Dichanthium* (De Wet & Harlan, 1970, Harlan & De Wet, 1963) in which overlapping patterns of morphological variation hamper the delimitation of species. This is at least partly caused by hybridization through facultative apomixis in spite of mechanisms that work against the gene flow, like geographical isolation, different flowering patterns, pollen-stigma incompatibility, (an)-euploidy and genic incompatibility (Harlan & Celarier, 1961). Further studies are needed to clarify the links between genetic and morphological similarities.

The agamic grass complexes consist mostly of perennials, while the *Brevivalvula* complex consists for a large part of annual taxa, with the exception of *P. setosum* and the relatively rare *P. atrichum*, in the more humid regions. Perennials have a different strategy of reproduction compared with annuals, because they can multiply and persist through vegetative proliferation, even when the generative reproduction is disturbed. Annuals, on the contrary, are completely dependent on viable seed produced at each generation, in order to perpetuate the population.

Obligate apomixis alone cannot be used in plant improvement programs. For breeders, obligate apomixis is an interesting characteristic, which can be manipulated when sexual types are discovered. New hybrids could be developed by crossing sexual female and apomictic male genotypes, stabilizing new desirable genetic characters (Hanna and Bashaw, 1987). Then, apomixis can be used to produce superior true-breeding hybrids, which would be easy and fast to reproduce, through open-pollination. However, in experiments crossing sexual and apomictic taxa, or testing molecular markers to recognize apomixis, one has to be careful with the choice of apomictic plant material. Often entire species are considered reproducing apomictically, this being obligately, while clones with different reproduction characteristics, the facultative apomicts, are not distinguished, although they exist. The clonal diversity is especially significant in *Pennisetum* section *Brevivalvula*. Moreover, using the highest ploidy level in a species in these crossing experiments, as is normally done, does not necessarily mean that the chances for obligate apomixis increase, as seen in this study.

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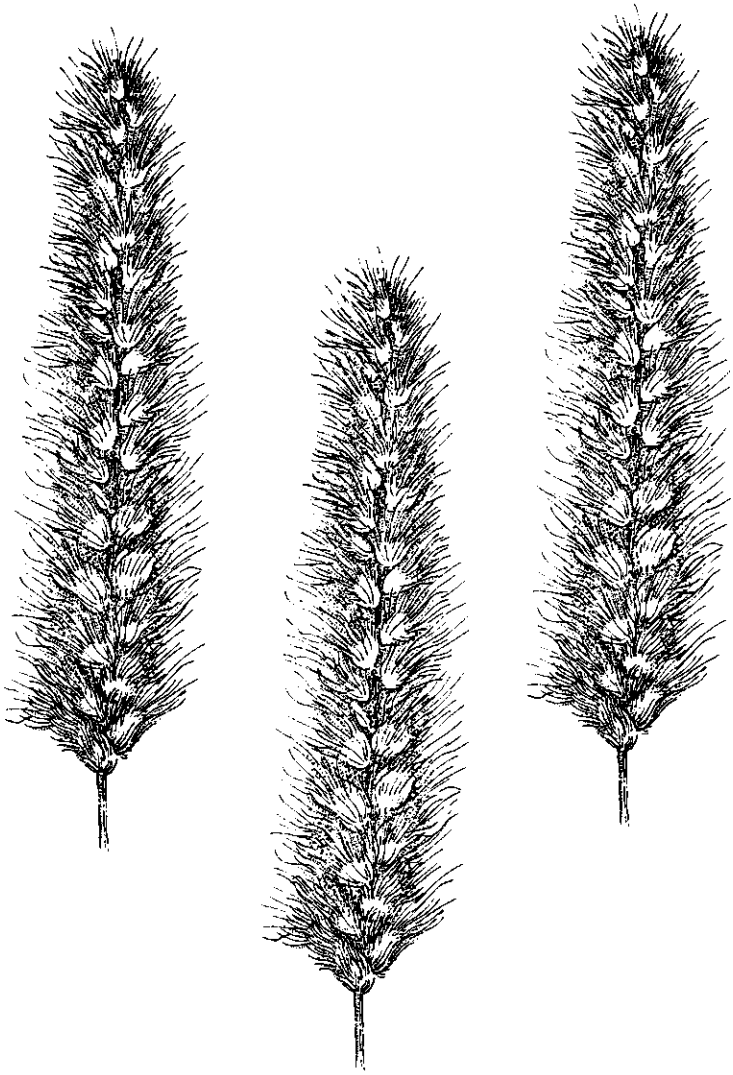


Pennisetum pedicellatum

Chapter 4

MORPHOLOGICAL VARIATION IN *PENNISETUM* SECTION *BREVIVALVULA*: PATTERNS OF SPECIES RELATIONSHIPS AND GEOGRAPHICAL DISTRIBUTION

Summary

Morphological variation among the 614 samples of the six species in *Pennisetum* section *Brevivalvula* was evaluated by principal component analysis. Nineteen characters, mainly from the inflorescence, revealed close morphological relationships among all species. *P. pedicellatum* was most differentiated and deviated also in its preference for the drier sahelian zone. The other species showed a large geographical overlap, but local dominance of different species was found. Ploidy levels were determined for the additional samples from Ivory Coast, revealing three more cytotypes in part of the species. The highest morphological and cytological variation was found in the mountainous savanna zone, which lies between the drier sahelian zone and the humid rain forest zones.

Introduction

An earlier morphological study in *Pennisetum* section *Brevivalvula* (Brunken, 1979b) was based on herbarium material selected in order to study the variation of the complex over the whole of tropical Africa. Three species were maintained as the result of this study: *P. hordeoides*, *P. pedicellatum*, in which two subspecies were recognized, *P. pedicellatum* ssp. *pedicellatum* and *P. pedicellatum* ssp. *unispiculum*, and *P. polystachion*, with three subspecies, *P. polystachion* ssp. *polystachion*, *P. polystachion* ssp. *atrichum*, and *P. polystachion* ssp. *setosum*. *P. subangustum* was not recognized as a taxon, but considered a synonym of *P. polystachion* ssp. *polystachion*.

The objectives of this study are in the first place to evaluate the morphological differences and similarities between the species of *Pennisetum* section *Brevivalvula*. Secondly, the geographical distribution patterns of the samples of the species and their cytotypes (at times called chromosomal taxa in this study for practical reasons) over four major vegetation zones in West Africa is evaluated as well. West Africa is supposed to be the center of diversity of the complex, because all species are known to occur here. The species are defined the same way as in the former chapters, in order not to loose any morphological variation formerly recognized in the section. The present study is different from Brunken's study because that study was a comparison of herbarium individuals originating from different populations in tropical Africa.

Material and methods

Sampling

To the 17 sites from southern Niger (2), Burkina Faso (6) and Benin (9), which have been used in Chapter 2, one more site from Burkina Faso and 19 sites from Ivory Coast (Table 1) have been added, resulting in 37 sites in total.

From each site the seeds of 20 individual plants were collected. Of these 740 samples, 614 were used, the others either did not germinate, did not flower or the ploidy level determination failed, and these plants were excluded from this study.

Table 1. The distribution of the sites from Ivory Coast over three major vegetation zones, and the geographical location of the sites. Zone I: undifferentiated sudanian woodland, Zone II: sudanian woodland with abundant *Isoberlinia*, Zone III: guineo-congolian mosaic of lowland rain forest and secondary grassland, and Zone IV: guineo-congolian rain forest (drier types). From Zone I no additional sites have been analyzed.

Site	Zone	Latitude	Longitude
56	II	10°17.18' N	04°54.42' W
58	II	09°35.90' N	05°15.56' W
59	II	09°30.20' N	05°31.05' W
61	II	09°30.27' N	06°18.08' W
62	II	09°36.11' N	06°46.41' W
66	II	09°30.84' N	07°33.34' W
67	II	09°04.24' N	07°45.35' W
68	II	08°38.41' N	07°27.84' W
72	III	07°42.44' N	07°26.18' W
75	IV	07°21.62' N	07°35.31' W
78	IV	06°57.67' N	07°28.75' W
81	IV	06°16.54' N	07°30.21' W
86	IV	05°21.57' N	07°17.13' W
89	IV	04°28.87' N	07°23.23' W
92	IV	04°46.07' N	06°40.44' W
97	IV	05°58.10' N	06°29.61' W
102	III	06°32.38' N	05°15.83' W
107	III	07°01.91' N	04°31.21' W
114	III	07°59.56' N	05°04.37' W
117	II	08°53.22' N	05°14.93' W

Estimation of ploidy levels by flow cytometry

DAPI flow cytometry was used for the determination of the ploidy levels of the *Pennisetum* samples originating from southern Niger, Burkina Faso and Benin (Chapter 2). The samples from Ivory Coast were analyzed at the Service de Cytométrie, Institut des Sciences Végétales UPR40, CNRS, 91198 Gif-sur-Yvette. In this case the AT dependent dye Hoechst 33342 was used to obtain the 2C nuclear DNA content. Samples from each species and ploidy level determined in the former study (Chapter 3) were used as reference. *Medicago sativa* ssp. x *varia* 'Rambler A2' was used as an internal standard, with 2n = 4x = 32.2C = 3.47 pg and 38.7% GC (Blondon et al., 1994).

Statistical and multivariate analysis

Most *Pennisetum* plants are daylight sensitive and their vegetative development, especially height and branching pattern, showed considerable differences when grown in Niger during different seasons. Therefore 19 reasonable stable characters were scored, including the life cycle, hairiness of the leaves, and morphological characters of the inflorescences (Table 2). Most of these characters have also been used by Brunken (1979b) in his study.

Table 2. Characters and character states used in the principal component analyses

1.	CYC	Lifecycle: annual (1) perennial (2)
2.	HAP	Hairiness of leaves and sheath: glabrous (0) hairy (1)
3.	PAL	Panicle length (mm)
4.	INN/P	Ratio: number of involucres/PAL
5.	TBL	Terminal bristle length (mm)
6.	BL/PH	Ratio: TBL/Height of bristle pubescence
7.	RAW	Rachis width (mm)
8.	NB6	Number of involucrum bristles longer than 6 mm
9.	SPN	Number of spikelets per involucrum
10.	PEL	Pedicel length (mm)
11.	SPL	Spikelet length (mm): if more than 1 spikelet was present, thelongest spikelet was measured
12.	SPW	Spikelet width (mm)
13.	BRN	Number of involucrum bristles
14.	NPB	Number of pubescent bristles
15.	LPB	Length of bristle pubescence (mm)
16.	PLL	Palea length (mm)
17.	PLW	Palea width (mm)
18.	SEL	Seed length (mm)
19.	SEW	Seed width (mm)

The characters were analyzed statistically using the JMP® statistical software package, version 3.4.1 (SAS Institute Inc., Cary, NC, USA). Tukey-Kramer HSD (honest significant difference) test (Tukey, 1953; Kramer, 1956) was applied to check for significant differences between the character means of all pairs of species, and the cytotypes of each species. With this multiple comparison test the confidence intervals of the group means are visualized with a comparison circles plot. Circles for means that are significantly different either do not intersect or intersect slightly only. The significance tests of all combinations of pairs are protected, and the Least Significant Difference (LSD) intervals become larger than the Student's t pairwise LSDs, thus differences between means become less significant.

Principal component analysis (PCA) was performed using JMP®, which is a method to establish independent grouping of populations. The analysis takes linear combinations of the standardized variables in a way that the first component has maximum variation, and the second component has next most variation, but is orthogonal to the first, etcetera.

The principal components are derived from an eigenvalue decomposition of the correlation matrix of the variables. The first two eigenvectors were used to obtain projections of the samples onto the principal axes, the other components gave no additional information.

Results

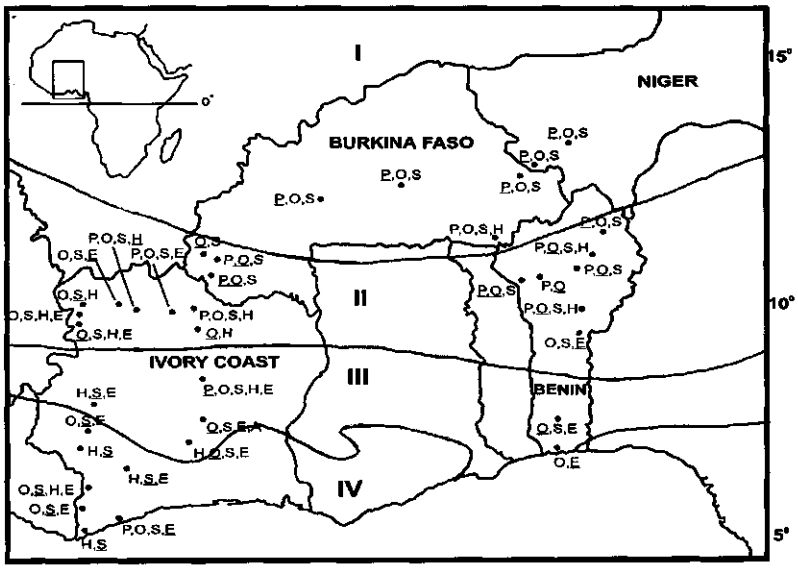
Distribution of ploidy levels over species and vegetation zones

Four ploidy levels were found in the 635 samples analyzed by flow cytometry: diploids, tetraploids, pentaploids and hexaploids.

The distribution of the samples of the species and ploidy levels over four large vegetation zones (White, 1986) is given in Figure 1a and 1b. Six sites were analyzed of Zone I, 18 of Zone II, seven of Zone III and six of Zone IV. Renno et al. (1995), see Chapter 2, already found 13 cytotypes distributed over 5 species, in southern Niger, Benin and Burkina Faso. During the collection trip to Ivory Coast one additional species, *P. atrichum*, and new cytotypes of *P. setosum* and *P. hordeoides* were collected. The cytotypes of these species are: tetraploid *P. atrichum*, tetra- and pentaploid *P. setosum* and pentaploid *P. hordeoides*.

Due to the biased sampling strategy, followed by the selection of sites with most variation for analysis, several species were found at each site. Local differences exist though for abundance of certain species. Table 3 summarizes the number of samples collected per chromosomal taxa, and per vegetation zone. *P. polystachion* (30%) and *P. subangustum* (28.5%) are the most common species in the samples, followed by *P. pedicellatum* (21.8%), *P. setosum* (13.4%) and *P. hordeoides* (6.2%), while *P. atrichum*(0.3%) is rare. Tetraploids are dominant(73%), followed by hexaploids(20%).

a.



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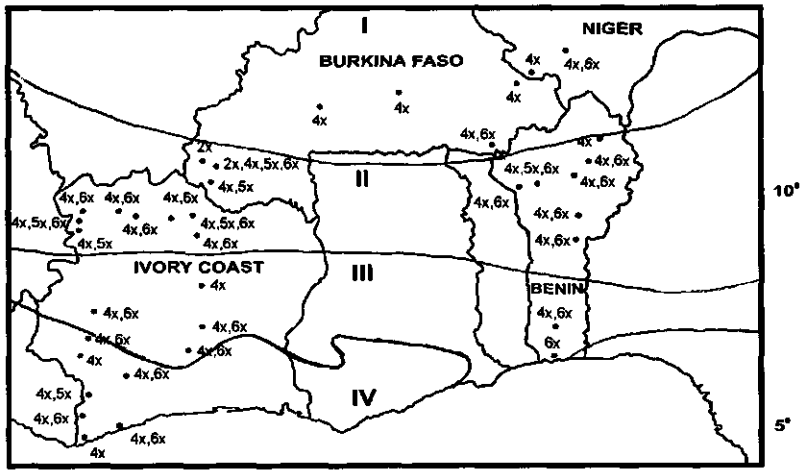


Figure 1a. The distribution of the species *P. pedicellatum* (P), *P. polystachion* (O), *P. subangustum* (S), *P. hordeoides* (H), *P. setosum* (E) and *P. atrichum* (A) over four vegetation zones (I, II, III and IV). See Table 1 for explanation of these zones. Each underlined letter indicates the predominant species at that particular site.
Figure 1b. The ploidy levels (2x, 4x, 5x, 6x) found at each site.

Table 3. The number of samples analyzed of each species of Pennisetum sction Brevivalvula, the distribution of the species over vegetation zones and over ploidy levels.See Table 1 for explanation of the zones

Taxon	Zone	Ploidy level				Total	%	Number of samples/site
		2x	4x	5x	6x			
P. atrichum	I							
	II							
	III		2			2		0.3
	IV							
Subtotal			2			**2**	**0.3**	**0.1**
P. hordeoides	I		2		1	3		0.5
	II		23			23		1.3
	III		6		1	7		1.0
	IV		4	1		5		0.8
Subtotal			35	1	2	**38**	**6.2**	**1.0**
P. pedicellatum	I		53	3	7	63		10.5
	II		47	4	12	63		3.5
	III		5			5		0.7
	IV		1		2	3		0.5
Subtotal			106	7	21	**134**	**21.8**	**3.6**
P. polystachion	I		22			22		3.7
	II	25	83	4	19	131		7.3
	III		23		1	24		3.4
	IV		4		3	7		1.2
Subtotal		25	132	4	23	**184**	**30.0**	**5.0**
P. subangustum	I		13			13		2.2
	II	4	57		2	63		3.5
	III		21		2	23		3.3
	IV		71		4	75		12.5
Subtotal		4	162		8	**174**	**28.3**	**4.7**
P. setosum	I							0
	II		1	2	27	30		1.7
	III		2		26	28		4.0
	IV		8		16	24		4.0
Subtotal			11	2	69	**82**	**13.4**	**2.2**
All species	I		90	4	8	101		
	II	29	211	10	60	310		
	III		59		30	89		
	IV		88		25	114		
Total		29	448	14	123	**614**		
%		4.7	73.0	2.3	20.0	100		

Two sites with diploids (4.7%) were found in vegetation zone II, and pentaploids (2.3%) are predominantly collected in this zone as well.

Only in *P. polystachion* all four ploidy levels were found: di-, tetra-, penta- and hexaploid; in *P. subangustum* di-, tetra-, and hexaploids were found; in *P. hordeoides, P. pedicellatum* and *P. setosum* tetra-, penta-, and hexaploids, and in *P. atrichum* only tetraploids.

The geographical distribution of the species and their cytotypes as found in Chapter 2, becomes more clear here. No new samples were added from the first vegetation zone while zone IV, in the south of Ivory Coast, was new. In Benin this zone was absent. *P. pedicellatum* occurs predominantly in zone I and II, with all three of its cytotypes, but the species has a significantly higher presence (χ^2 = 115.15; d.f. = 4; P<0.01) in zone I than the other species. All *P. polystachion* cytotypes occur in zone II. The species has a significantly higher presence in this zone (χ^2 = 53.89; d.f. = 4; P<0.01) than the other species, except *P. hordeoides*. Tetraploids occur regularly in the other zones as well. *P. hordeoides* is predominantly tetraploid, and occurs in all zones. It has a significantly higher presence in zone II (χ^2 = 4.27; d.f. = 2; P<0.01) than *P. subangustum* and *P. setosum*. *P. subangustum* is predominantly tetraploid, and has a significant higher presence (χ^2 = 10.73; d.f. = 3; P<0.05) in zone IV than the other species, except *P. setosum*. It is also regularly present in the other three zones. *P. setosum* is perennial, predominantly hexaploid, and occurs significantly more in zone III (χ^2 = 37.65; d.f. = 4; P<0.01) than the other species except *P. hordeoides*, and also in zone IV (χ^2 = 128.13; d.f. = 4; P<0.01) except for *P. subangustum*. The species was missing completely from the driest zone I. The other perennial species, *P. atrichum*, was found in zone III only.

Significant differences exist between part of the chromosomal taxa. Neither *P. atrichum*, nor the pentaploids have been included in the comparison, because the number of samples is too low. If tetra- and hexaploids of the other five species are compared, *P. setosum* has significantly more hexaploids (χ^2 = 234.42; d.f. = 4; P<0.01) than the other species. *P. hordeoides* and *P. subangustum* consist mainly of tetraploids, and differ significantly from *P. pedicellatum* and *P. polystachion* (χ^2 = 14.31; d.f. = 3; P<0.01) for having less hexaploids. *P. subangustum* has also significantly less diploids than *P. polystachion* (χ^2 = 15.52; d.f. = 1; P<0.01).

Statistical analysis

The mean and standard deviation of each character were determined for each chromosomal taxon (Table 4). The means were tested with Tukey-Kramer HDS-test for significant differences. Distinctive characters are (1) the life cycle (CYC), which is perennial for *P. setosum* and *P. atrichum*, and annual for the others; (2) the number of

Table 4. Mean (M) and Standard Deviation (SD) per character and per species and cytotype. For character abbreviations see Table 2.

Species and cytotype	Nb. of plants		CYC	HAP	PAL	INN/P	TBL	BL/PH	RAW	NB6	SPN	PEL	SPL	SPW	BRN	NPB	LPB	PLL	PLW	SEL	SEW
P. atrichum																					
4x	**2**	**M**	**2**	**1**	**107.5**	**2.36**	**12.0**	**0**	**0.8**	**1.0**	**1**	**0**	**3.5**	**1.5**	**13.0**	**0**	**0.1**	**1.8**	**0.8**	**1.4**	**0.7**
		SD	**0**	**0**	**10.6**	**0.42**	**1.6**	-	**0.1**	**0.0**	**0**	-	**0.0**	**0.1**	**1.4**	-	**0.0**	**0.1**	**0.0**	**0.1**	**0.0**
P. hordeoides																					
4x	35	M	1	0.6	98.7	3.03	9.1	0	0.8	1.6	1	0	3.2	1.3	8.9	0	0.1	2.0	0.7	1.5	0.6
		SD	0	0.3	13.6	0.73	2.1	-	0.1	0.9	0	-	0.3	0.2	2.9	-	0.0	0.2	0.1	0.2	0.0
5x	1		1	0.0	116.0	4.46	8.5	0	1.1	1.0	1	0	3.0	1.4	6.0	0	0.1	1.6	0.6	1.3	0.6
		SD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6x	2	M	1	0.5	115.0	2.47	12.3	0	0.8	2.5	1	0	3.8	1.2	9.0	0	0.1	2.1	0.7	1.4	0.6
		SD	0	1.1	12.7	1.35	4.9	-	0.0	2.1	0	-	0.9	0.1	5.7	-	0.0	0.3	0.1	0.1	0.7
total	**38**	**M**	**1**	**0.6**	**100.0**	**3.04**	**9.3**	**0**	**0.8**	**1.6**	**1**	**0**	**3.3**	**1.3**	**8.8**	**0**	**0.1**	**2.0**	**0.7**	**1.5**	**0.6**
		SD	**0**	**0.3**	**14.0**	**0.78**	**2.3**	-	**0.1**	**0.9**	**0**	-	**0.4**	**0.2**	**2.9**	-	**0.0**	**0.2**	**0.1**	**0.2**	**0.7**
P. pedicellatum																					
4x	106	M	1.1	0.4	107.2	0.99	18.7	2.56	0.8	13.7	2.5	1.2	4.2	1.5	51.3	41.0	2.3	2.1	0.8	1.7	0.7
		SD	0	0.1	24.7	0.42	4.5	0.45	0.1	5.5	0.9	0.8	0.5	0.2	20.1	1.9	0.6	0.2	0.1	0.2	0.0
5x	7	M	1	0.7	101.7	0.68	18.4	2.50	0.8	14.4	2.9	1.8	4.5	1.4	50.0	38.7	2.7	2.1	0.9	1.6	0.8
		SD	0	0.4	25.3	0.22	5.6	0.41	0.1	3.6	0.7	0.5	0.8	0.1	25.0	8.8	0.7	0.2	0.1	0.1	0.1
6x	21	M	1	0.7	121.0	0.96	21.5	2.52	0.9	16.1	3.0	1.7	4.3	1.5	54.5	43.2	2.7	2.3	0.8	1.7	0.7
		SD	0	0.2	16.9	0.40	3.4	0.37	0.1	6.8	0.8	1.0	0.6	0.1	25.0	4.6	0.5	0.3	0.1	0.2	0.0
total	**134**	**M**	**1**	**0.5**	**109.1**	**0.97**	**19.1**	**2.57**	**0.8**	**14.1**	**2.6**	**1.4**	**4.2**	**1.5**	**51.7**	**41.6**	**2.4**	**2.2**	**0.8**	**1.7**	**0.7**
		SD	**0**	**0.5**	**24.1**	**0.41**	**4.5**	**0.49**	**0.1**	**5.7**	**0.9**	**0.8**	**0.6**	**0.2**	**21.1**	**8.8**	**0.6**	**0.3**	**0.1**	**0.2**	**0.1**

Table 4 continued.

Species and cytotype	Nb. of plants		CYC	HAP	PAL	INN/P	TBL	BL/PH	RAW	NB6	SPN	PEL	SPL	SPW	BRN	NPB	LPB	PLL	PLW	SEL	SEW
P. polystachion																					
2x	25	M	1.2	0.9	131.3	2.76	17.8	5.42	0.9	3.9	1	0	4.4	1.4	30.0	13.0	0.9	2.2	0.7	1.6	0.7
		SD	0.1	0.2	32.7	0.71	2.1	1.30	0.1	1.4	0	-	0.5	0.1	7.7	3.9	0.3	0.2	0.1	0.2	0.0
4x	132	M	1.1	0.6	110.6	2.47	16.0	3.52	0.8	6.1	1	0	3.5	1.3	24.5	12.6	1.3	2.1	0.7	1.6	0.6
		SD	0.0	0.1	20.1	0.66	2.6	0.92	0.1	1.8	0	-	0.4	0.2	8.0	1.6	0.4	0.2	0.1	0.2	0.0
5x	4	M	1.0	0.5	116.5	3.14	15.2	2.92	0.8	5.0	1	0	3.5	1.3	20.3	9.0	1.2	1.9	0.7	1.4	0.6
		SD	0.0	0.7	43.2	0.60	2.6	0.26	0.2	1.8	0	-	0.3	0.3	4.8	22.3	0.2	0.3	0.1	0.2	0.1
6x	23	M	1.4	0.7	116.7	2.58	16.4	3.11	0.8	6.4	1	0	3.5	1.4	24.2	12.8	1.7	2.0	0.7	1.5	0.6
		SD	0.1	0.3	21.1	0.76	2.8	0.85	0.1	1.6	0	-	0.4	0.2	7.0	3.9	0.4	0.2	0.1	0.2	0.0
total	**184**	**M**	**1.2**	**0.7**	**114.3**	**2.54**	**16.3**	**3.75**	**0.8**	**5.8**	**1**	**0**	**3.6**	**1.3**	**25.1**	**12.6**	**1.3**	**2.1**	**0.7**	**1.6**	**0.6**
		SD	**0.1**	**0.7**	**23.6**	**0.69**	**2.6**	**1.29**	**0.1**	**1.9**	**0**	**-**	**0.5**	**0.2**	**8.0**	**22.3**	**0.4**	**0.2**	**0.1**	**0.2**	**0.1**
P. setosum																					
4x	11	M	1.9	0.7	138.3	2.61	17.1	3.38	0.8	6.9	1	0	3.5	1.5	19.7	11.4	1.3	2.1	0.7	1.5	0.6
		SD	0.1	0.3	30.4	0.60	4.4	0.55	0.1	1.5	0	-	0.4	0.1	6.1	6.9	0.3	0.2	0.1	0.2	0.1
5x	2	M	1.5	0.0	92.5	2.48	17.3	4.32	0.9	5,0	1	0	3.3	1.6	19.0	7.5	1.1	1.9	0.7	1.5	0.6
		SD	1.6	0.0	10.6	0.01	3.8	1.97	0.1	0,0	0	-	0.4	0.0	2.8	6.3	0.1	0.1	0.0	0.1	0.7
6x	69	M	2.0	0.7	152.1	2.66	18.9	4.51	0.9	7.6	1	0	3.6	1.4	21.2	10.3	1.3	2.1	0.7	1.6	0.6
		SD	0.0	1.1	29.5	0.71	4.0	1.21	0.1	1.6	0.2	-	0.4	0.2	6.2	2.2	0.4	0.3	0.1	0.2	0.0
total	**82**	**M**	**1.9**	**0.7**	**149.0**	**2.65**	**18.7**	**4.37**	**0.9**	**7.4**	**1**	**0**	**3.5**	**1.4**	**21.0**	**10.4**	**1.3**	**2.1**	**0.7**	**1.6**	**0.6**
		SD	**0.1**	**1.1**	**30.7**	**0.68**	**4.0**	**1.28**	**0.1**	**1.6**	**0.2**	**-**	**0.4**	**0.2**	**6.1**	**6.9**	**0.4**	**0.3**	**0.1**	**0.2**	**0.7**
P. subangustum																					
2x	4	M	1.0	1.0	130.8	2.98	12.0	4.01	1.0	1.5	1	0	4,0	1.5	25.0	12.3	0.9	2.2	0.8	1.6	0.8
		SD	0.0	0.7	32.7	0.80	1.8	0.51	0.1	0.6	0	-	0.1	0.1	8.1	15.3	0.2	0.1	0.1	0.1	0.1
4x	162	M	1.1	0.7	103.3	2.59	10.3	2.84	0.8	2.7	1	0	3.2	1.4	19.2	10.7	1.1	2.0	0.7	1.5	0.6
		SD	0.0	0.1	20.3	0.76	2.0	0.73	0.1	1.7	0	-	0.3	0.1	7.4	1.6	0.3	0.3	0.1	0.2	0.0
6x	8	M	1.4	0.6	114.6	2.69	10.7	2.97	0.8	2.9	1	0	3.2	1.4	17.7	9.0	1.1	2.1	0.7	1.5	0.6
		SD	0.2	0.4	20.7	0.75	2.8	0.90	0.1	2.1	0	-	0.2	0.1	6.5	8.8	0.2	0.2	0.1	0.2	0.1
total	**174**	**M**	**1.1**	**0.7**	**104.4**	**2.61**	**10.4**	**2.90**	**0.8**	**2.7**	**1**	**0**	**3.3**	**1.4**	**19.2**	**10.7**	**1.1**	**2.0**	**0.7**	**1.5**	**0.6**
		SD	**0.2**	**0.7**	**21.0**	**0.75**	**2.0**	**0.85**	**0.1**	**1.7**	**0**	**-**	**0.3**	**0.1**	**7.4**	**15.3**	**0.3**	**0.2**	**0.1**	**0.2**	**0.1**

spikelets (SPN), which is one to five in *P. pedicellatum*, and one in the other species. If in a *P. pedicellatum* sample only one spikelet per involucre is found, it still differs from the other species (3) by having a short pedicel (PEL): at least one of the spikelets in *P. pedicellatum* has a distinctive pedicel, the spikelets of the other species are sessile. *P. pedicellatum* plants with one shortly pedicelled spikelet are considered a different subspecies by Brunken (1979b), *P. pedicellatum* ssp. *unispiculum*. It is supposed to be a hybrid between *P. pedicellatum* and *P. polystachion*. Finally, (4) the length of the bristle pubescence (LPB) distinguishes *P. atrichum* and *P. hordeoides* from the other species, because it is (almost) absent in those species. In the other four species LPB is an overlapping character.

For the remaining characters, significant differences between the samples of the species rather indicate tendencies than true differences between the species. In Table 5 all significant differences found between species are listed. *P. atrichum*, with only two observations, was obscuring differences between the other species because of its large standard error. *P. setosum* has relatively longer panicles (PAL) than the other species. *P. pedicellatum* has a lower ratio of number of involucres and panicle length (INN/P), a higher number of involucrum bristles (BRN), a higher number of involucrum bristles exceeding 6 mm (NB6), and longer bristle pubescence (LPB). *P. subangustum* and *P. hordeoides* have shorter terminal bristles (TBL) and a lower number of involucrum bristles exceeding 6 mm (NB6) than the other species. *P. hordeoides* also had a lower total number of involucrum bristles (BRN) than the other species. Figure 2 (a to e) illustrates for six of these characters the significant differences that are found between the means of the species.

In Table 6 the significant differences (at 1% confidence level) are given between the cytotypes of each species. The diploid samples of *P. polystachion* have a significantly higher mean than the tetraploids for the characters PAL, TBL, BL/PH, RAW, SPL, BRN, PLL, and INN/P, and a significantly higher mean than the hexaploids for the characters BL/PH, SPL, BRN and PLL. The diploid samples had a significantly lower mean than the tetra- and hexaploids for the characters NB6 and LPB, while the tetraploids had a significantly lower mean for these characters than the hexaploids. The diploids of *P. polystachion* in general show the larger and more compact inflorescences, and not, as could be expected, the hexaploids. Within *P. subangustum* the same pattern is visible, but for less characters. The diploid samples had a significant higher mean than the tetraploids for the characters PAL, BL/PH, RAW, and SPL, and a significantly higher mean than the hexaploids for the character SPL. Significant differences between the tetra- and hexaploid *P. pedicellatum* samples were found for the means of the characters PAL, TBL, SPW, LPB and PLL, indicating a tendency for larger dimensions in these characters in hexaploid *P. pedicellatum*. Hexaplois *P. setosum* has a significantly higher mean than the tetraploids only for the character BL/PH. Figure 3 illustrates the variation between the chromosomal taxa for three of these characters: PAL, TBL, and NB6.

Table 5. Morphological characters significantly different between species with Tukey-Kramer HSD ($p<0.01$). For character abbreviations see Table 2.

	P. pedicellatum	*P. polystachion*	*P. subangustum*	*P. hordeoides*	*P. setosum*	*P. atrichum*
P. pedicellatum		INN/P,TBL,BL/PH NB6,SPL,SPW BRN,LPB,PLW SEL,PEL	RAW,INN/P,TBL NB6,PLL,SEL SPL,SPW,BRN LPB,PLW,SPN PEL	INN/P,TBL,NB6 SEL,SPL,SPW BRN,PLW,SPN PEL,LPB	CYC,PAL,INN/P NB6,SPL,BRN SPW,SEL,PEL BL/PH	CYC,BRN,NB6 SPW,PEL,LPB
P. polystachion			PAL,TBL,BL/PH NB6,SPL,BRN LPB	PAL,INN/P,TBL NB6,SPL,BRN LPB	CYC,PAL,TBL BL/PH,NB6,SPW	–
P. subangustum				INN/P,BRN LPB	CYC,PAL,TBL NB6,RAW,SPL LPB,BL/PH	–
P. hordeoides					CYC,PAL,TBL, NB6,SPW,BRN LPB	–
P. setosum						–
P. atrichum						

Table 6. Morphological characters significantly different for cytotypes per species, with Tukey-Kramer HDS (p<0.01).

P. polystachion	2x	4x	6x
2x		PAL,TBL,BL/PH RAW,SPL,BRN PLL,NB6,LPB	BL/PH,SPL,BRN PLL,NB6,LPB
4x			NB6,LPB
P. subangustum	2x	4x	6x
2x		PAL,BL/PH RAW,SPL	SPL
P. setosum		4x	6x
4x			BL/PH
P. pedicellatum		4x	6x
4x			TBL,SPW,LPB

Principal component analysis

Two PCA's have been performed on the morphological data set. The first PCA is on the complete data set with 614 plants (Fig. 4), where the first two eigenvectors accounted for 30.0% respectively 14.6% of the variation. The factor loadings are rather low, but are reasonable considering mostly characters from the inflorescence are used. Factor 1 had the highest positive loading of characters NB6, BRN, SPN, and LPB, and the highest negative loading of character INN/P. Factor 2 had the highest positive loading of the characters PAL, CYC and TBL (Table 6).

The first eigenvector largely separates the samples indicated as *P. pedicellatum* from the other species, the group with one sessile spikelet. This result confirms the results of Brunken (1979b), who analyzed 177 herbarium specimens representing all 6 species originating from tropical Africa. In the *P. pedicellatum* group, no subgroups can be distinguished on the basis of ploidy level or site (results not shown). The samples of *P. pedicellatum* ssp. *unispiculum,* the morphologically intermediate plants

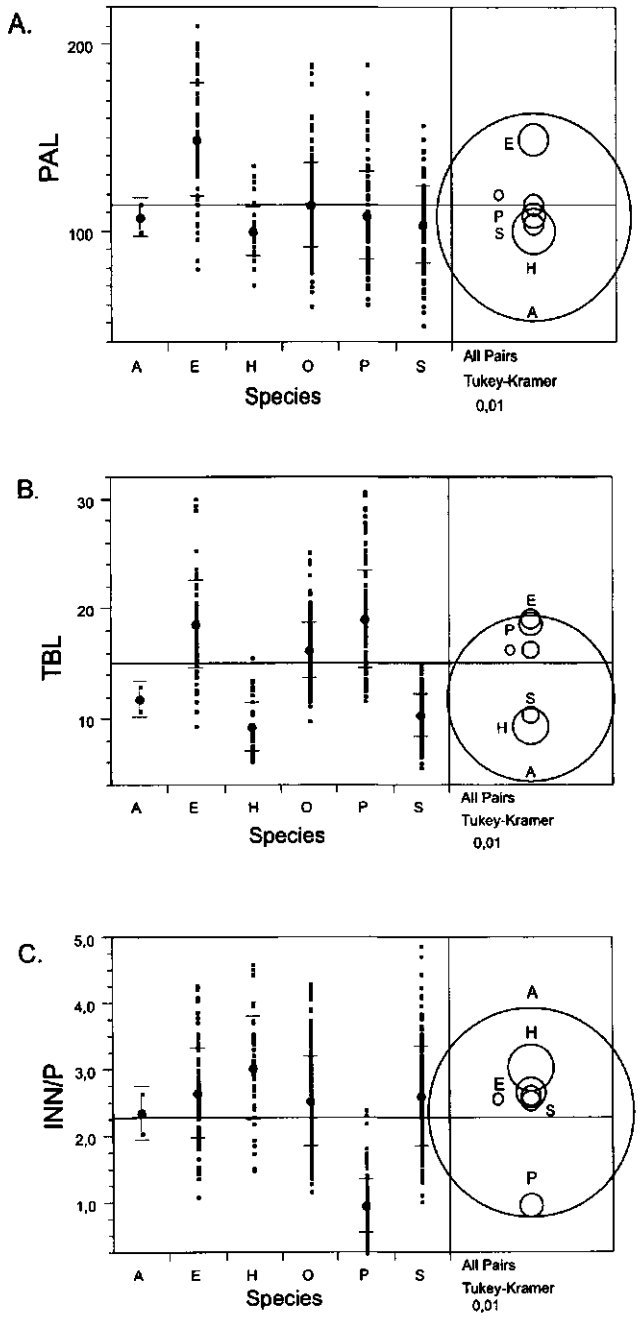
between *P. pedicellatum* and *P. polystachion*, which were recognized first by Brunken (1979b), are indicated separately.

In order to examine patterns of variation in the sessile spikelet group more closely, the pedicelled plants were removed from the data set and a second PCA was performed, allowing a better scattering of the remaining plants. The second PCA (Fig. 5) shows one large group in which the different taxa are indicated by symbols. On a more or less horizontal scale, from left to right, one can distinguish: *P. hordeoides*, *P. subangustum*, *P. polystachion* and *P. setosum*. Both *P. atrichum* samples are with the samples of *P. subangustum*.

The values for TBL, NB6, BL/PH, PAL and CYC are increasing from left to right. *P. hordeoides* and *P. subangustum* are both species with mostly narrow inflorescences. *P. hordeoides* has few and scabrous involucrum bristles, while *P. subangustum* has more and hairy involucrum bristles, but both characters show intermediate forms. *P. subangustum* and *P. polystachion* show a very large overlap, making it often very difficult to distinguish between them. In general *P. polystachion* samples have longer and larger inflorescences. *P. setosum*, the perennial species, has the longest and largest inflorescences, and is perennial.

The second principal component is composed of eigenvectors in which all species show a large plasticity, causing a stretching of the group.

When the ploidy levels of the samples are plotted in the second PCA (Fig. 6), tetraploids cover most of the left and middle part of the cloud, while the right side is covered mainly by hexaploids. The diploids occur primarily in the region where tetraploids and hexaploids overlap, and the pentaploids are scattered among the tetraploids.


A.
PAL
200
100
A E H O P S
Species
All Pairs
Tukey-Kramer
0,01
E
O
P
S
H
A
B.
TBL
30
20
10
A E H O P S
Species
All Pairs
Tukey-Kramer
0,01
E
P
O
S
H
A
C.
INN/P
5,0
4,0
3,0
2,0
1,0
A E H O P S
Species
All Pairs
Tukey-Kramer
0,01
A
H
E
O
S
P

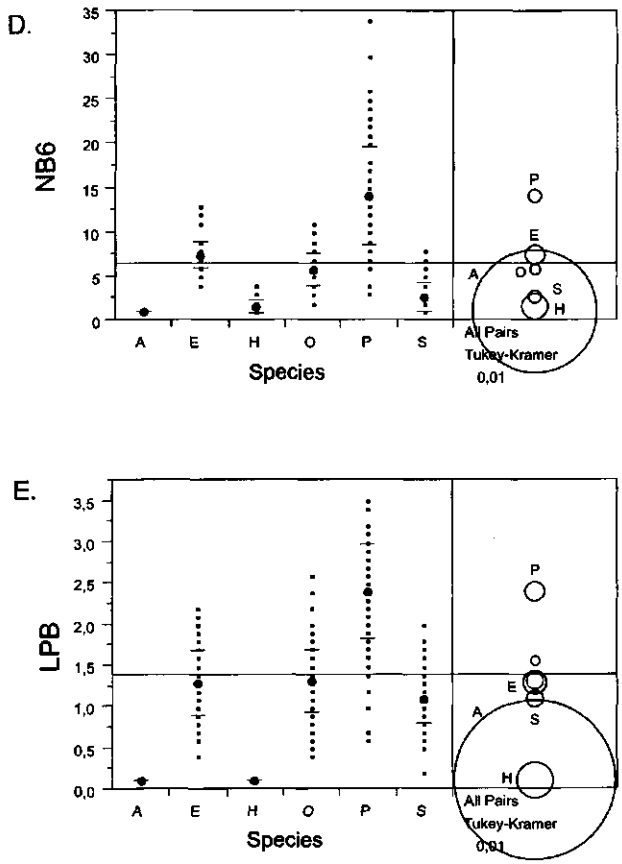


Figure 2. Significant differences between species visualized for the following characters: a. PAL, b. TBL, c. INN/P, d. NB6, and e. LPB. At the left side of the figure the range of the character for each species is given. The black dots indicate the mean for each species, and the two horizontal lines indicate the standard error. At the right side of the figure the circles indicate the 1% confidence interval for each species. If the angle between two overlapping circles is larger than 90^0, the species are not significantly different.

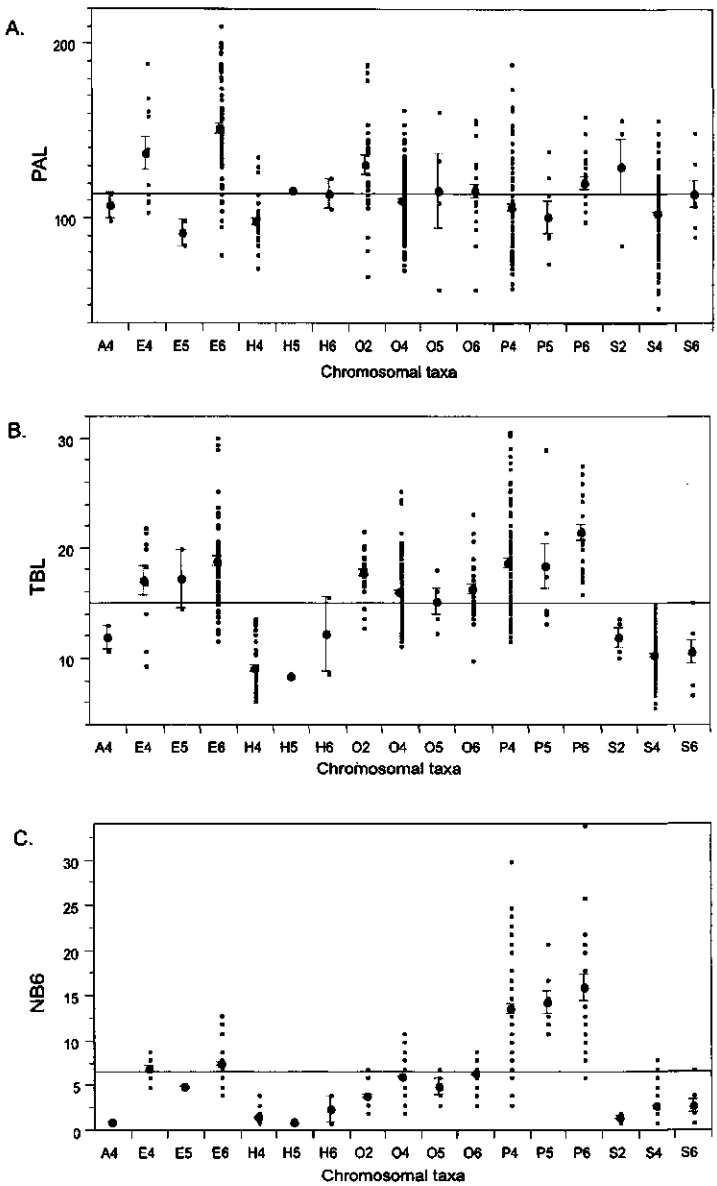


Figure 3. Variation between cytotypes of species visualized for the following characters: a. PAL, b. TBL and c. NB6. The black dots indicate the mean for each species, and the horizontal lines indicate the standard deviation.

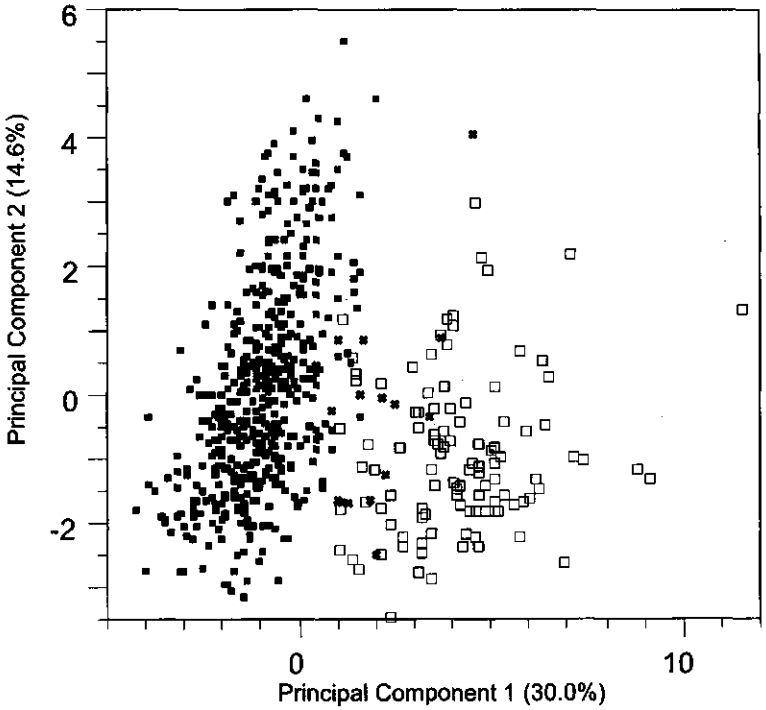


Figure 4. PCA of the samples of *Pennisetum* section *Brevivalvula*:
P. pedicellatum (□), *P. pedicellatum* ssp. *unispiculum* (X), and the other species of the section (▪).

Table 7. Highest character loadings of both principal component analyses on the first 2 eigenvectors.

PCA of all species (614 samples)				
Eigenvector 1		Eigenvector 2		
NB6	0.389	PAL	0.480	
BRN	0.361	CYC	0.389	
SPN	0.358	BL/PH	0.384	
LPB	0.354			
SPL	0.316			
INN/P	-0.312			
Variation 30.0%		Variation 14.6%		Total 44.6%
PCA of all species except *P. pedicellatum* (487 samples)				
Eigenvector 1		Eigenvector 2		
TBL	0.440	PLL	0.489	
NB6	0.401	PLW	0.457	
BL/PH	0.348	SEL	0.412	
PAL	0.336	BRN	-0.389	
CYC	0.308	HAP	0.331	
Variation 22.8%		Variation 13.9%		Total 36.7%

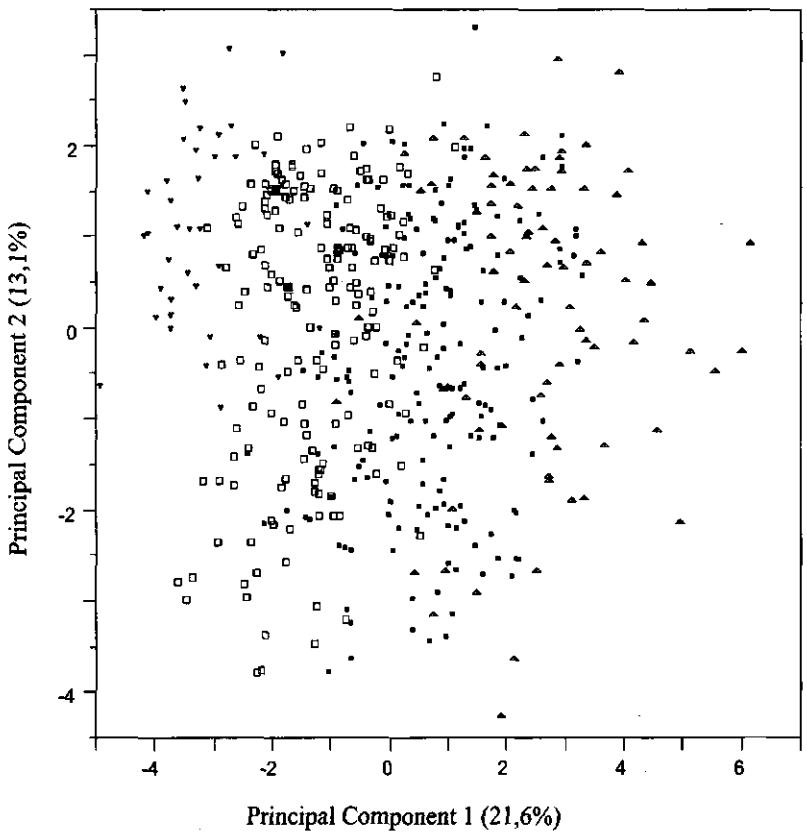


Figure 5. PCA of the samples of *Pennisetum* section *Brevivalvula* without *P. pedicellatum*: *P. hordeoides* (Y), *P. subangustum* (□), *P. polystachion* (▪), *P. setosum* (△) and *P. atrichum* (■).

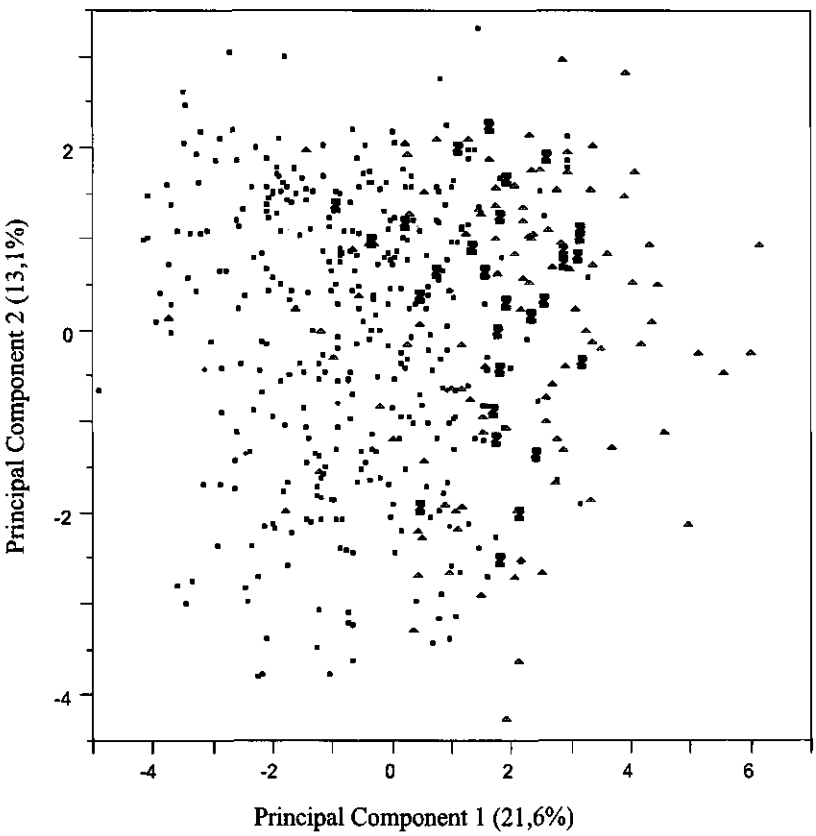


Figure 6. Identical PCA as Figure 5, but the symbols indicate the ploidy levels: diploids (**Z**), tetraploids (▪), pentaploids (+) and hexaploids (△).

Discussion

In many polyploid agamic complexes hybridization is a common feature, obscuring morphological boundaries between taxa. Well-known examples where these events occur in grasses are the *Botriochloa-Dichanthium* complex (Harlan et al., 1962; Harlan, 1963; De Wet & Harlan, 1966, 1970), *Panicum* (Assienan & Noirot, 1995) and polyploid *Poa* (Clausen, 1954, 1961). Usually, taxa are distinct geographically, ecologically and/or cytologically, and are recognized at species, subspecies, or variety level. Where species are sympatric, the patterns become disturbed, and sometimes

difficult to discern at all. In *Pennisetum* section *Brevivalvula* most species are sympatric in West Africa, indicating that this is the center of diversity of the section. As can be seen from the statistical analysis of the morphological characters and the results of the principal component analysis, morphological overlap between all taxa exists. *P. pedicellatum* is the morphologically and geographically most differentiated species, but intermediate forms between *P. pedicellatum* and *P. polystachion* occur, and are recognized by Brunken (1979a) as the putative hybrid *P. pedicellatum* ssp. *unispiculum*. Eighteen of these putative hybrids have been found among the 127 *P. pedicellatum* samples analyzed in this study. Most hybrids are tetraploids, but one hexaploid was found as well. They occur often in small groups in populations dispersed over the main part of the distribution area of the species, vegetation zones I and II. Two *P. pedicellatum* ssp. *unispiculum* samples from different sites were among those with variable progeny (P651 and P1111, Chapter 3). The other 16 hybrid samples were not analyzed in that study. Other samples with variable progeny originated from the same sites as well, a *P. pedicellatum* sample with two spikelets (P649) in one case and a *P. polystachion* sample (O1017) in the other, forming a strong indication for these hybridization events.

The second PCA, without *P. pedicellatum*, shows a continuous pattern between four of the other taxa, if the two *P. atrichum* samples are not taken into account. Brunken (1979a) found, on the basis of herbarium material, that *P. hordeoides* formed a distinct group, and he kept the taxon at the species level. In this study there is some overlap only with *P. subangustum*, indicating that *P. hordeoides* belongs also to the *P. polystachion* complex. The herbarium specimens used in Brunken's study, individual samples collected from different populations, probably do not represent the morphological variation at population level well. Different cytotypes cannot be distinguished either from herbarium specimen. The different chromosomal taxa in this study showed some tendencies, when the number of samples was large enough. The hexaploids had higher means for certain characters than the tetraploids, while the diploids had for some characters higher means than the hexaploids.

Other, mostly vegetative differences exist between the species, but these were not studied here because the variation of these characters was very high during different growing seasons. *P. hordeoides* and *P. subangustum* are both usually small and slender plants with mostly narrow inflorescences. In general, *P. polystachion* plants are bigger, profusely branching, and with longer and larger inflorescences. The perennial *P. setosum* is also robust, but has most of its leaves, which are much longer than the other species, at the basis, sign of its perennial life cycle, and rather few branched inflorescences. *P. setosum* and the other perennial species, *P. atrichum*, are also different from the other species, because the color of the inflorescences is often yellowish, or reddish-brown, while the color for the other species varies from white to violet to purple.

A plausible reason for finding only few *P. atrichum* samples among our samples could be that the species is rare in the region. Brunken (1979b) used herbarium

specimens mainly from the central part of Africa, only two out of 17 specimens came from the very West of West Africa, indicating a possible sparse distribution of this taxon in the sampled region. In his principal component analysis the *P. atrichum* samples grouped close to *P. setosum*, while in this study both samples grouped closer to the other side of the morphological range, *P. hordeoides*, but no conclusions can be drawn because of the low number of samples.

On the basis of the results found in this study one can conclude that the species are morphologically very similar, but individual samples can often be differentiated on the basis of a few distinctive characters. Most of the taxa in the complex recognized by Brunken are validated by this study. *P. subangustum* though, reduced to a synonym of *P. polystachion*, should become a taxon, because it forms the morphological link between *P. hordeoides* and *P. polystachion*, and because typical samples for that taxon can be recognized readily. This reduction of *P. subangustum* as a synonym is in contrast with the new classification by Brunken of *P. pedicellatum* ssp. *unispiculum*, which is a less coherent group than *P. subangustum*.

Pennisetum section *Brevivalvula* could be an evolutionary young group, compared to other agamic grass complexes like the *Bothriochloa-Dichanthium* complex, in which many of the valid species are good taxa, and introgression occurs mostly when *Bothriochloa intermedia*, the compilospecies (Harlan & De Wet, 1963), is involved. Speciation in agamic complexes is slow because sexual diploids as well as residual sexuality in part of the polyploids facilitate hybridization events among the taxa, obscuring species boundaries (Stace, 1975). Speciation will only occur when a taxon finds its own ecological niche and gene exchange with other taxa becomes (almost) disrupted. This could be the case with *P. pedicellatum*, which has its main distribution in the dry sahelian zone. In the second vegetation zone, where the complex is most diverse, *P. pedicellatum* has to compete with the other taxa, and can only dominate locally. *P. hordeoides* has been observed to form monomorphic patches in this zone, on old farmland. Towards the humid south, *P. setosum* and *P. subangustum* take over this pattern of locally dominant morphotypes, but mixed populations exist as well. *P. polystachion*, *P. pedicellatum* and *P. setosum* are also taking advantage of man-made environments by colonizing ruderal areas in other continents. Since only a part of the gene pool is migrating in these cases, certain morphological or genetically distinct forms can stabilize in an environment without competition from other forms and become a new taxon.

Another scenario for the complex is, especially at places where diploids are present, that continuous hybridization causes a constant reshuffling of the genotypes, so that no speciation occurs, but populations stay a mixture of different morphotypes, ploidy levels and genotypes. The second vegetation zone could well be such a region for *Pennisetum* section *Brevivalvula*.

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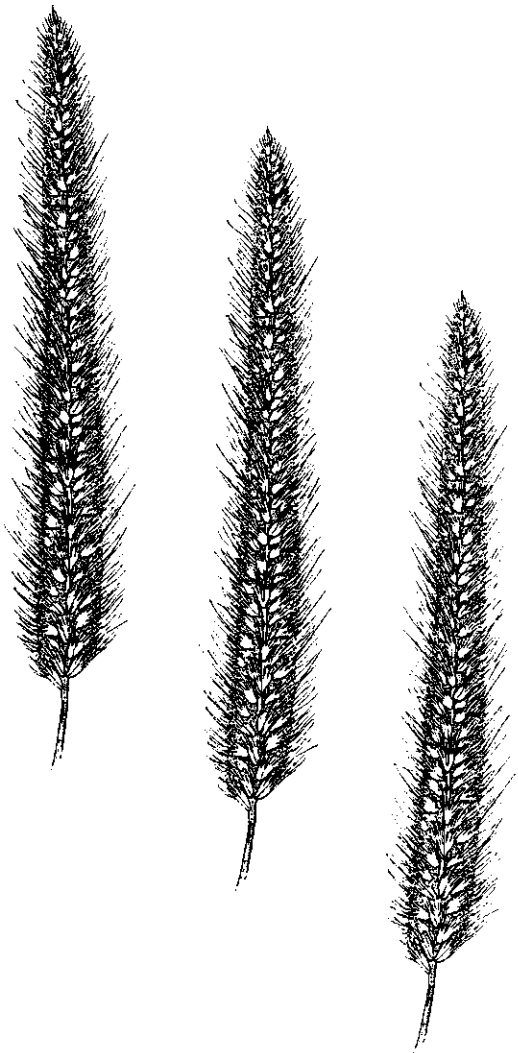


Pennisetum polystachion

Chapter 5

PENNISETUM SECTION *BREVIVALVULA*: PATTERNS OF GENOTYPIC VARIATION IN WEST AFRICA

Summary

Genotypic variation between 635 samples of *Pennisetum* section *Brevivalvula* originating from West Africa was evaluated using isozyme electrophoresis. A total of 146 different 5-locus genotypes, from combinations of 26 alleles, has been found. Of these genotypes 57% occurred in only 13% of the samples. The remaining 43% was shared between different species, chromosomal taxa or geographical zones and regions. The most common chromosomal taxa, tetraploid *P. pedicellatum*, *P. polystachion*, *P. subangustum* and *P. hordeoides*, and hexaploid *P. setosum*, shared many of their genotypes. In *P. polystachion* all 26 alleles were found, including 6 rare alleles. Most of the 20 remaining alleles were found among the other species of the section. The mean number of alleles (A) and the mean observed heterozygosity (Ho) were not significantly different between the species, nor was there a clear geographic effect. A and Ho were significantly lower in the diploid cytotypes of *P. polystachion* and *P. subangustum* than in the polyploid species. The expected heterozygosity (He) was only determined for the diploids, and was not significantly different from Ho. In the area where the diploids were found, also most alleles and genotypes were found. Multivariate correspondence analysis showed that all species, ploidy levels or geographical areas had significantly different proportions of the alleles. The allelic patterns found in the samples of the morphological species in *Pennisetum* section *Brevivalvula* indicate as well that they belong to the same gene pool.

Introduction

All species in the agamic species complex of *Pennisetum* section *Brevivalvula* overlap in their morphological characters (Chapter 4). *P. pedicellatum* is most differentiated in West Africa, both morphologically and ecologically, but the other species, *P. polystachion*, *P. subangustum*, *P. hordeoides*, *P. setosum* and *P. atrichum* can be recognized as well, despite the existence of many morphologically intermediate samples. One to four cytotypes have been discovered in the samples of each of the species of the section (Chapter 2 and 4). The tetraploid cytotype is the predominant cytotype in most species, followed by the hexaploid cytotype. *P. setosum* is mainly a hexaploid species. Pentaploids have been found in low numbers in most species, while diploid, sexual reproducing plants have been found in *P. polystachion* and *P.*

subangustum. Brunken (1979) made a revision of the section, based on herbarium material from tropical Africa. He decided to classify some species as subspecies, and one species as a synonym, because of morphological overlapping characters. In this study the morphological taxa in the section were recognized at species level in order to preserve all morphological polymorphism.

Molecular markers might be able to clarify the relations between the morphological species. Enzyme electrophoresis has been shown to be very useful for the exploration of genetic diversity in closely related species (Brown, 1978; Crawford, 1989). This marker has been chosen in the present study also for practical reasons. The first objective of the study was to analyze the genotypic variation in the samples of *Pennisetum* section *Brevivalvula* in order to determine whether the isozyme patterns were taxonomically or geographically distributed. The second objective was to comprehend the effects of sexual and apomictic reproduction systems in the complex.

Material and methods

Plant material

The same 614 samples were used as in Chapter 4, with 21 additional plants. These 635 plants were distributed over 37 sites, and these sites were geographically grouped into four large vegetation zones, that are horizontally orientated, or grouped into three vertically orientated regions. The following vegetation zones have been distinguished (illustrated in Figure 1 of Chapter 4): Zone I: undifferentiated sudanian woodland, Zone II: sudanian woodland with abundant *Isoberlinia*, Zone III: guineo-congolian mosaic of lowland rain forest and secondary grassland, and Zone IV: guineo-congolian rain forest (drier types). The following regions have been distinguished: the northern region (N), which equals Zone I, the eastern region (E), which equals zone II and III of Benin and the western region (W), which equals Zone II, III, and IV of Ivory Coast, and the West of Burkina Faso. Ploidy level analyses were described in Chapter 2 and 4.

Enzyme polymorphisms

Each of the 635 samples was analyzed by starch gel electrophoresis for five enzymatic systems, that were selected for known polymorphism, and because they could be analyzed simultaneously. The same techniques and staining schedules were used as in Chapter 2 and 3 (Wendel & Weeden, 1989). The five enzymatic systems are: phosphoglucose mutase (PGM), E.C. 5.4.2.2.; glucose-6-phosphate isomerase (GPI), E.C. 5.3.1.9.; phosphogluconate dehydrogenase (PGD), E.C. 1.1.1.44; isocitrate dehydrogenase (IDH), E.C. 1.1.1.41 (42); and malate dehydrogenase (MDH) E.C. 1.1.1.37. A genotype in this study is formed by the combination of different alleles at 5 putative loci.

Data analysis

Statistics

The alleles for all isozymes were scored for each of the 635 samples. The samples were grouped into different categories: species (6), chromosomal taxa (17), ploidy levels (4), vegetation zones (4), and regions (3). The genotypic variation of the samples was calculated using the following parameters: the mean number of alleles per locus (A), and the observed heterozygosity (Ho = heterozygotes/homo- and heterozygotes).

For the diploids the expected heterozygosity under Hardy-Weinberg equilibrium across all loci (He) was estimated by the F statistic of Wright, with F_{is} = 1 - Ho/He (Wright, 1965). F_{is} is positive if He > Ho (deficit of heterozygotes), and negative if Ho > He (surplus of heterozygotes). The program GENETIX (Belkhir et al., 1996) calculates the theoretical F_{is} for each locus, and tests for significant differences by comparison to the observed F_{is}.

The homo- and heterozygotes for each ploidy level per locus were analyzed with the JMP® Statistical software package, version 3.4.1 (SAS Institute Inc., Cary, NC, USA). Tukey-Kramer HSD (honest significant difference) test (Tukey, 1953; Kramer, 1956) is a multiple comparison test, and was applied to check for significant differences among the number of alleles of the different ploidy levels. The significance tests of all combinations of pairs are protected, and the Least Significant Difference (LSD) intervals become larger than the Student's t pairwise LSDs, thus differences between means become less significant.

Correspondence Analysis

Correspondence analysis (Benzécri, 1973) is similar to principal component analysis (PCA), but is especially adapted to qualitative data like allelic presence and absence. It differs from PCA in using χ^2 distance rather than the Euclidian distance, and the possibility to represent projections of the variables and categories on the same scatterplot. Similarities of this method with PCA are mainly: (1) how many independent factors explain the diversity, (2) what part of the diversity is explained and (3) the meaning of factors (factor loading). The different categories in which the samples are grouped are: species (6), ploidy levels (4), vegetation zones (4) and regions (3).

The categories with high loadings in a particular scatterplot were tested with χ^2 for significant differences.

Results

Enzymic polymorphism

One putative locus with its allelic variations was established for each enzyme system. As to the polyploids, only the band position was considered, not their intensity, so the allelic frequencies could not be calculated.

The five loci used to characterize the genetic variation of the genotypes are polymorphic with a total of 26 alleles and 37 allelic combinations (Fig. 1):

- *Mdh*: two homodimeric isozymes corresponded to the allelic variations at one locus, two distinct genotypes were observed.
- *Pgd*: four homodimeric isozymes corresponded to the allelic variations at one locus, three distinct genotypes were observed.
- *Pgm*: five monomeric isozymes corresponded to the allelic variations at one locus, 15 distinct genotypes were observed.
- *Idh*: six homodimeric isozymes corresponded to the allelic variations at one locus, six distinct genotypes were observed.
- *Gpi*: nine homodimeric isozymes corresponded to the allelic variations at one locus, 11 distinct genotypes were observed.

Genotypic relationships between chromosomal taxa

Comparison of genotypes

The percentage of individuals belonging to different chromosomal taxa (= taxon x cytotype) or to different ploidy levels, with the allelic proportions from five isozyme loci, is given in Table 1a. These percentages do not represent allelic frequencies.

The maximum number of alleles is found in *P. polystachion* (26 alleles), followed by *P. pedicellatum*, *P. subangustum*, and *P. setosum* (19, 19 respectively 18 alleles), *P. hordeoides* (16 alleles) and finally *P. atrichum* (only 8 alleles, probably through lack of genitors). Many rare alleles are found only in *P. polystachion*: *Pgd* 94 and 106, *Pgi* 40, 60, 95, and 120.

The alleles *Pgm* 92 and *Idh* 116 were found in *P. polystachion*, and also in *P. pedicellatum* (*Pgm* 92), or in *P. subangustum* (*Idh* 116). Of the other alleles *Idh* 76 is completely absent from *P. hordeoides*, and *Pgm* 108 from *P. pedicellatum*. The remaining alleles are found in all species.

In total, 146 different 5-locus genotypes were found in the 635 genitors. Of these 146 genotypes, 57.5% (13.2% of the samples) was found either only once (48.6%), or more than once but in a single chromosomal taxon (8.9%). The remaining 42.5% of the genotypes (or 86.8% of the samples) was shared between several chromosomal taxa: 18.5% was shared between two chromosomal taxa only, 12.3% between 3 chromosomal taxa, 3.4% between 4 and 5 chromosomal taxa each, 2.1% between 6

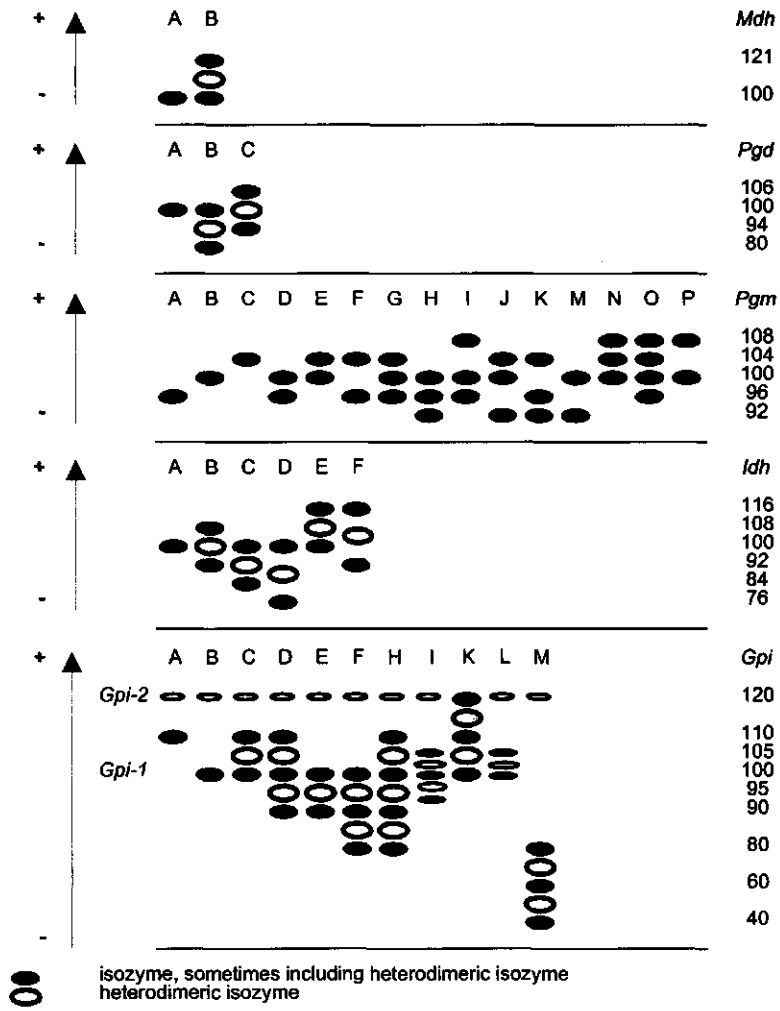


Figure 1. Zymograms of the 5 enzyme systems, Mdh, Pgd, Pgm, Idh and Gpi, with 26 alleles. The numbers at the right indicate the names of the alleles, while the capital letters above each zymogram indicate the names of the allelic pattern.

chromosomal taxa, and 0.5% between 7, 8, 9, or 11 chromosomal taxa. Based on these results, the samples seem to belong to the same gene pool.

Figure 2 shows the patterns of relationships that exist between the different chromosomal taxa. The first number next to each chromosomal taxon is the number of genetic connections the taxon has with other chromosomal taxa. All chromosomal taxa are at least twice connected with other chromosomal taxa. Tetraploid *P. subangustum* (S4), *P. pedicellatum* (P4), *P. polystachion* (O4), hexaploid *P. setosum* (E6) and diploid *P. polystachion* (O2) share genotypes with most other chromosomal taxa, while tetraploid *P. subangustum* (S4), *P. pedicellatum* (P4), and *P. polystachion* (O4) have the highest percentages of genotypes shared between them. Hexaploid *P. hordeoides* (H4), pentaploid *P. setosum* (E5) and tetraploid *P. atrichum* (A4) also share a large part of their genotypes, despite the low numbers of samples analyzed in these chromosomal taxa. The second number next to each chromosomal taxon is the number of genotypes found only in that chromosomal taxon. The chromosomal taxa S4, P4, O4, and H4 have most of those unique genotypes.

Allelic diversity and heterozygosity

In Table 2a the mean number of alleles (A) and the mean level of observed heterozygosity (Ho) of the loci are given for each species and chromosomal taxon. The polyploid taxa all have a higher mean number of alleles (A = 1.4 to 1.7), than the diploid chromosomal taxa (A = 1.2). The mean heterozygosity (Ho) is highest for *P. hordeoides* (Ho = 0.42), followed closely by *P. polystachion*, *P. pedicellatum* and *P. subangustum* (Ho = 0.40, 0.39 respectively 0.38), while the lowest numbers are found in *P. setosum* and *P. atrichum* (Ho = 0.32 respectively 0.30). The diploids are characterized by a lower level of heterozygosity (Ho = 0.15-0.19) than the polyploids.

Allelic diversity of the ploidy levels

When only ploidy levels are compared (Table 1b), most alleles are found in the tetraploids (24 alleles), followed by hexaploids (20 alleles), while di- and pentaploids have few alleles (10 respectively 12 alleles).

The mean number of alleles and the mean heterozygosity (Table 2b), is lower in the diploids (A = 1.2; Ho = 0.19) than in the polyploids, but the heterozygosity level in the pentaploids is lower than in the tetra- or hexaploids (Ho = 0.25, compared to Ho = 0.40).

For each ploidy level the numbers of alleles present in the heterozygotes (two, three or four alleles) were compared to the single allele present in the homozygotes, for each locus (Figure 3 a to e). The diploids were only compared for two alleles. For *Mdh*, *Pgd* and *Idh* a maximum of two alleles were counted for heterozygotes in each sample, in *Pgm* and *Gpi* up to four alleles were counted. In *Mdh* and *Pgd* no significant differences were found with Tukey-Kramer HSD-test between homo- and heterozygotes of all ploidy levels, due to the low number of heterozygotes in those

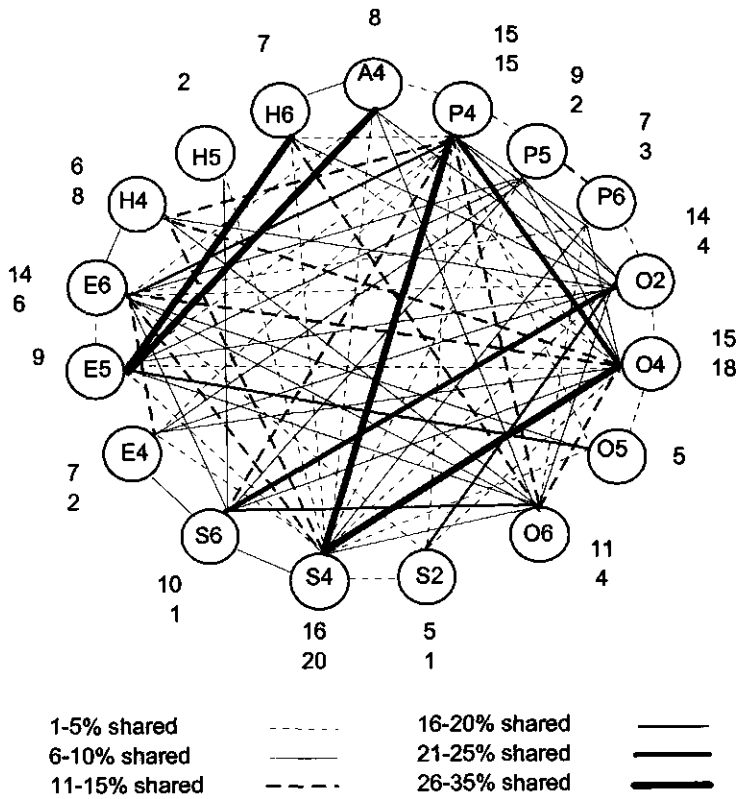


Figure 2. Diagram of the 17 chromosomal taxa with shared 5-locus genotypes.
The circled symbols indicate the chromosomal taxa: P = *P. pedicellatum*, O = *P. polystachion*, S = *P. subangustum*, E = *P. setosum*, H = *P. hordeoides*, and A = *P. atrichum*, and 2 = diploid, 4 = tetraploid, 5 = pentaploid, and 6 = hexaploid cytotypes. Of the numbers next to each chromosomal taxon, the first one indicates the number of chromosomal taxa genotypes are shared with. The second number indicates the number of genotypes only found in that chromosomal taxon.

Table 1. The percentage of samples analyzed per allele: (a) for the 17 different chromosomal taxa, and (b) for the four ploidy levels

(a)

	P. atrichum	*P. setosum*				*P. hordeoides*				*P. polystachion*				
Ploidy level	4x	4x	5x	6x	mean	4x	5x	6x	mean	2x	4x	5x	6x	mean
Locus														
Mdh														
100	1	1	1	1	1	1	1	1	1	1	1	1	1	1
121	**0**	0	0	0.03	**0.03**	0.21	0	0	**0.20**	0	0.09	0	0.31	**0.10**
Pgd														
80	**0**	0.10	0	0	**0.01**	0.08	1	0	**0.10**	0.08	0.01	0	0.04	**0.02**
94	**0**	0	0	0	**0**	0	0	0	**0**	0	0	0	0.04	**0.005**
100	1	1	1	1	1	1	0	1	0.98	1	1	1	1	1
106	**0**	0	0	0	**0**	0	0	0	**0**	0	0	0	0.04	**0.005**
Pgm														
92	**0**	0	0	0	**0**	0	0	0	**0**	0	0.01	0	0	**0.007**
96	**0**	0.80	0.50	0.55	**0.58**	0.28	0	0.5	**0.28**	0.12	0.46	0.83	0.69	**0.46**
100	**1**	1	1	1	1	0.97	1	1	**0.97**	0.92	1	1	1	**0.99**
104	**0.50**	0.50	0	0.72	**0.69**	0.54	1	0.5	**0.55**	0.24	0.44	0.50	0.69	**0.45**
108	**0**	0.40	0.50	0.28	**0.30**	0.03	0	0	**0.03**	0	0.03	0.50	0.08	**0.05**
Idh														
76	**0**	0.10	0	0.01	**0.02**	0	0	0	**0**	0	0.06	0	0	**0.04**
84	**0**	0	0	0.05	**0.04**	0.21	0	0	**0.20**	0.04	0.12	0	0.50	**0.15**
92	**0**	0.40	0	0.01	**0.06**	0.10	0	0	**0.09**	0	0.18	0	0	**0.13**
100	**1**	0.60	1	0.99	**0.95**	0.90	1	1	**0.91**	1	0.82	1	1	**0.87**
108	**0**	0.40	0	0.01	**0.06**	0.10	0	0	**0.09**	0	0.18	0	0	**0.13**
116	**0**	0	0	0	**0**	0	0	0	**0**	0	0.02	0	0	**0.01**
Gpi														
40	**0**	0	0	0	**0**	0	0	0	**0**	0	0.01	0	0	**0.007**
60	**0**	0	0	0	**0**	0	0	0	**0**	0	0.01	0	0	**0.007**
80	**0**	0.10	0	0.01	**0.02**	0.23	0	0	**0.21**	0	0.12	0	0	**0.09**
90	**0.50**	0.80	0.50	0.55	**0.58**	0.51	0	0	**0.47**	0	0.46	0.83	0.46	**0.41**
95	**0**	0	0	0	**0**	0	0	0	**0**	0	0.02	0	0	**0.01**
100	**1**	1	1	1	1	1	1	1	1	0.64	0.99	1	1	**0.95**
105	**0**	0	0	0.01	**0.009**	0	0	0	**0**	0	0.02	0	0	**0.01**
110	**0.50**	0.30	0.50	0.44	**0.42**	0.69	1	1	**0.71**	0.92	0.59	0.50	0.65	**0.64**
120	**0**	0	0	0	**0**	0	0	0	**0**	0	0.01	0	0	**0.007**
Nb. of alleles	**8**	14	9	12	**18**	16	7	8	**16**	10	24	10	14	**26**

Table 1 continued.

	P. pedicellatum				*P. subangustum*				(b) Ploidy levels			
Ploidy level	4x	5x	6x	mean	2x	4x	6x	mean	2x	4x	5x	6x
Locus												
Mdh												
100	1	1	1	**0,98**	1	1	1	1	1	1	1	1
121	0.02	0	0	**0.02**	0	0.04	0.29	**0.05**	0	0.08	0.06	0.09
Pgd												
80	0.05	0	0.05	**0.05**	0.25	0.04	0	**0.04**	0.1	0.03	0	0.02
94	0	0	0	**0**	0	0	0	**0**	0	0	0	0.008
100	1	1	1	1	1	1	1	1	1	1	1	1
106	0	0	0	**0**	0	0	0	**0**	0	0	0	0.008
Pgm												
92	0.05	0	0	**0.04**	0	0.01	0	**0.009**	0	0.02	0	0
96	0.62	0.43	0.65	**0.62**	0.25	0.50	0.14	**0.48**	0.14	0.50	0.56	0.57
100	0.90	0.86	1	**0.91**	1	0.96	1	**0.96**	0.93	0.96	0.94	1
104	0.44	0.71	0.80	**0.50**	0	0.43	0.57	**0.43**	0.21	0.45	0.56	0.72
108	0	0	0	**0**	0	0.04	0.29	**0.06**	0	0.03	0.25	0.19
Idh												
76	0	0.29	0	**0.01**	0	0.03	0	**0.03**	0	0.03	0.13	0.01
84	0.23	0	0.40	**0.24**	0	0.18	0.14	**0.17**	0.03	0.17	0	0.20
92	0.02	0	0.15	**0.04**	0	0.10	0	**0.09**	0	0.11	0	0.03
100	0.98	1	0.85	**0.96**	1	0.90	1	**0.91**	1	0.89	1	0.97
108	0.02	0	0.15	**0.04**	0	0.10	0	**0.09**	0	0.11	0	0.03
116	0.01	0	0	**0,01**	0	0.01	0	**0.009**	0	0.01	0	0
Gpi												
40	0	0	0	**0**	0	0	0	**0**	0	0.002	0	0
60	0	0	0	**0**	0	0	0	**0**	0	0.002	0	0
80	0.07	0	0.15	**0.08**	0	0.33	0	**0.31**	0	0.19	0	0.03
90	0.38	0.14	0.35	**0.37**	0	0.51	0.29	**0.49**	0	0.47	0.44	0.48
95	0	0	0	**0**	0	0	0	**0**	0	0.01	0	0
100	1	1	1	1	0.50	1	1	**0.99**	0.62	1	1	1
105	0	0	0	**0**	0	0	0	**0**	0	0.01	0	0.01
110	0.55	0.71	0.70	**0.58**	0.75	0.45	0.86	**0.47**	0.90	0.53	0.63	0.55
120	0	0	0	**0**	0	0	0	**0**	0	0.002	0	0
Nb. of alleles	14	10	14	**16**	8	19	12	**19**	10	24	12	20

Table 2. Mean number of alleles (A) and mean observed heterozygosity (Ho), based on the number of samples, (a) for species and chromosomal taxa, and (b) for ploidy levels.

(a)

Species and Cytotypes	Number of Samples	% different Genotypes	A	Ho
P. atrichum				
4x	2	100	1.3	0.30
P. setosum	87		1.5	0.32
4x	10	70	1.7	0.23
5x	2	10	1.4	0.30
6x	75	37	1.5	0.33
P. hordeoides	43		1.5	0.42
4x	40	65	1.6	0.42
5x	1	100	1.6	0.6
6x	2	100	1.2	0.40
P. polystachion	194		1.5	0.40
2x	25	36	1.2	0.19
4x	138	41	1.5	0.41
5x	6	83	1.6	0.40
6x	25	60	1.7	0.53
P. pedicellatum	130		1.5	0.39
4x	103	41	1.5	0.38
5x	7	71	1.4	0.34
6x	20	70	1.7	0.46
P. subangustum	179		1.4	0.38
2x	4	100	1.2	0.15
4x	168	39	1.5	0.38
6x	7	86	1.5	0.43

(b)

Ploidy level	Number of Samples	% different Genotypes	A	Ho
2x	29	38	1.2	0.19
4x	461	28	1.5	0.40
5x	16	75	1.5	0.25
6x	129	37	1.5	0.40

loci. For *Idh* there were significantly less heterozygotes in the diploids than in the tetraploids (5%). For *Pgm* and *Gpi* pentaploids did have up to three alleles, tetra- and hexaploids up to four alleles. Significant less two-allele heterozygotes were found for the diploids compared to tetra- or hexaploids (1%); significantly more three-allele heterozygotes were found (5%) in penta- and hexaploids compared to tetraploids, and significantly more four-allele heterozygotes are found in hexaploids (5%) than in tetra- and pentaploids.

For the diploids, the samples of *P. polystachion* and *P. subangustum* taken together, heterozygotes were present in the three loci *Pgd*, *Pgm* and *Gpi*. The observed and theoretical F_{IS} were not significantly different for each of the three loci (observed F_{IS} = -0.04 (*Pgd*), 0.22 (*Pgm*), and -0.13 (*Gpi*); $p < 0.05$). Thus, the hypothesis cannot be rejected that for these loci the diploids of these two species are reproducing panmictically, and act like one population.

Geographical organization of the isozyme diversity

Geographical distribution of the alleles

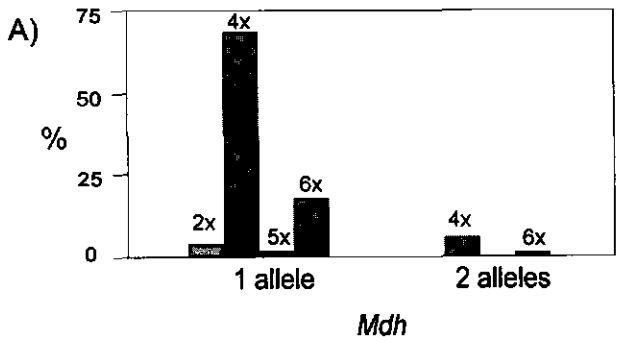
The proportion of alleles found in each of the 37 different sites is given in Table 3. The sites are grouped into four horizontally orientated vegetation zones or three vertical orientated regions in order to observe possible distribution patterns of the alleles.

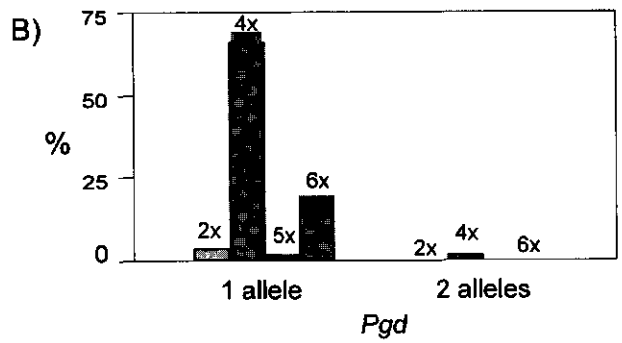
The maximum number of alleles is found in Zone II (26 alleles), followed by the other 3 zones (18 alleles in Zone III, 17 alleles in Zone IV and 16 alleles in Zone I). When the three regions are compared, the eastern region (E) combines more alleles (24) than the western region (W) or the northern region (N), 20 respectively 16 alleles. When the results of these two geographical groups are combined, it becomes clear that the eastern side of zone II has most allelic diversity. This is the zone where the diploid populations are present.

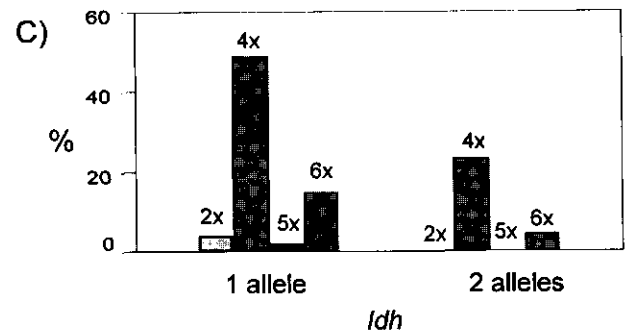
The six rare alleles *Pgd* 94 and 106, and *Gpi* 40, 60, 95 and 120 are only found in zone II. *Pgd* 80, *Pgm* 108, *Idh* 76, and *Gpi* 105 are not found in vegetation zone I (which equals the northern region); *Pgd* 80 and *Pgm* 92 are not found in zone III; and *Pgm* 92, *Idh* 116 and *Gpi* 105 are not found in zone IV. The relatively rare alleles *Idh* 116 and *Gpi* 105 were not found in the eastern region.

Geographical distribution of the 5-locus genotypes

The distribution of the 146 genotypes over the four vegetation zones or over the three regions, is summarized in Table 4. In the zones I and II more genotypes were found that have a restricted distribution (on average 3 respectively 3.3 of these genotypes per site) than in the other two zones (2 respectively 1.7 of these genotypes on average per site). If the distinction is made according to region, more genotypes with a restricted distribution were found in the eastern region, in Benin, with 4.1 genotypes per site, than in the other two regions.


A)
75
50
%
25
0
4x
2x
5x
6x
4x
6x
1 allele
2 alleles
Mdh


B)
75
50
%
25
0
4x
2x
5x
6x
2x
4x
6x
1 allele
2 alleles
Pgd


C)
60
40
%
20
0
4x
2x
5x
6x
4x
2x
5x
6x
1 allele
2 alleles
Idh

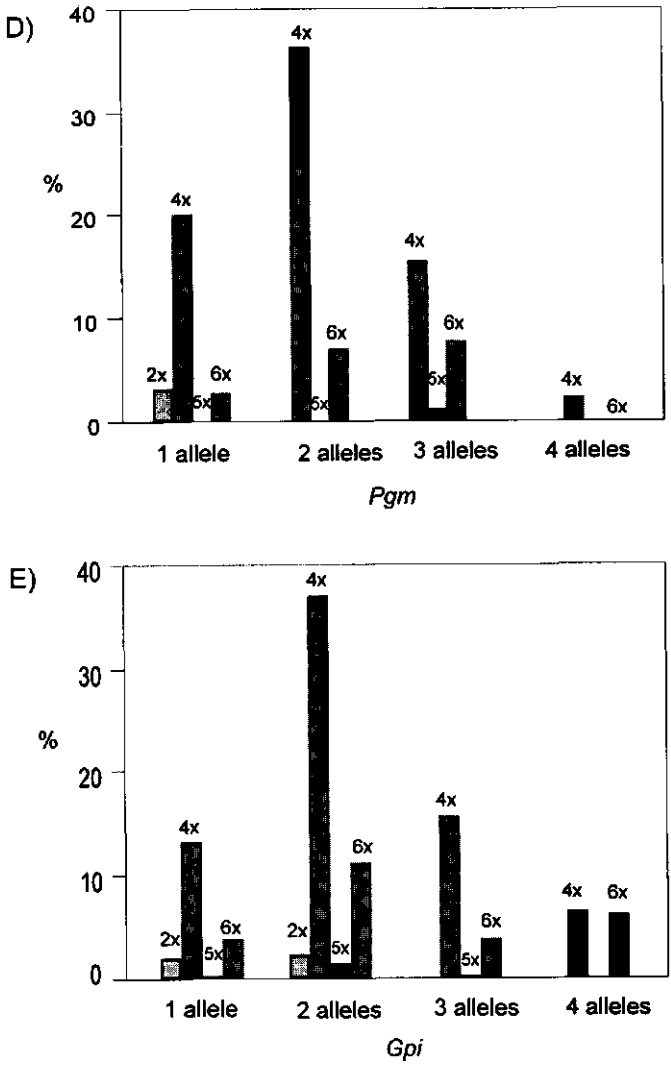


Figure 3. Histograms of the percentage of homozygotes (1 allele) and heterozygotes (2, 3 or 4 alleles) per ploidy level (2x, 4x, 5x, and 6x) found in the samples, for each locus: A. *Mdh*, B. *Pgd*, C. *Idh*, D. *Pgm*, and E. *Gpi*.

Table 3. The percentage of samples analyzed per allele, (A) for the 37 different sites, and (B) for the four vegetation zones and three regions

(A)

	Sites																										
	1	2	3	9	13	19	21	23	26	27	29	30	31	37	38	43	48	56	58	59	61	62	66	67	68	72	75
Locus																											
Mdh																											
100	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
121	0	0.06	0	0.06	0	0	0	0	0.13	0.13	0.15	0	0	0	0.05	0.50	0.06	0.05	0	0	0.20	0	0.25	0.06	0.18	0.41	0
Pgd																											
80	0	0	0	0	0	0	0.15	0	0	0	0.15	0.15	0.11	0.09	0	0	0	0	0.05	0.07	0.05	0	0.06	0	0	0	0.21
94	0	0	0	0	0	0	0	0	0	0.10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	1	1	1	1	1	1	1	1	1	0.90	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
106	0	0	0	0	0	0	0	0	0	0.10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pgm																											
92	0	0.11	0	0.12	0	0.06	0	0	0	0	0	0	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96	0.67	0.44	0.94	0.76	0.57	0.12	0.15	0.47	0.63	0.50	0.60	0.65	0.56	0	0.42	0.67	0.29	0.26	0.05	0.50	0.70	0.71	0.81	0.56	0.47	0.29	0.53
100	0.80	1	0.89	0.76	0.70	1	0.90	1	1	1	1	0.95	0.94	1	1	1	1	1	0.95	1	1	1	1	1	1	1	1
104	0.47	0.61	0.06	0.59	0.74	0.35	0.25	0.60	0.63	0.69	0.75	0.75	0.67	0.64	0.79	0.22	0.06	0.26	0.36	0.43	0.35	0.88	0.25	0.56	0.29	0.29	0.16
108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.06	0.18	0	0	0.07	0	0	0.06	0.44	0.06	0.24	0
Idh																											
76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0.16	0.09	0.14	0	0	0	0	0	0	0
84	0.33	0.28	0.22	0.41	0.48	0.06	0.05	0.20	0.31	0.38	0.10	0.35	0	0.55	0.37	0.06	0	0	0.09	0.14	0.25	0	0	0.06	0	0	0.32
92	0.13	0.11	0	0.06	0	0	0	0.20	0.06	0	0.10	0	0.06	0.09	0	0	0	0	0.14	0.07	0.30	0.06	0.25	0.22	0.53	0	0.42
100	0.87	0.89	1	0.94	1	1	1	0.80	0.94	1	0.90	1	0.94	0.91	1	1	1	1	0.86	0.93	0.70	0.94	0.75	0.78	0.47	1	0.58
108	0.13	0.11	0	0.06	0	0	0	0.20	0.06	0	0.05	0	0.06	0.09	0	0	0	0	0.14	0.07	0.30	0.06	0.25	0.22	0.53	0	0.42
116	0	0.06	0	0	0	0	0	0	0.13	0	0.05	0	0	0	0	0.11	0	0	0	0	0	0	0	0	0	0	0
Gpi																											
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.10	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.10	0	0
80	0.07	0	0.06	0	0.13	0.06	0	0	0.13	0.19	0	0.15	0.11	0.18	0	0.33	0	0	0	0.29	0.05	0.06	0.13	0.17	0.18	0.41	0.05
90	0.13	0.33	0.61	0.29	0.26	0.06	0	0.40	0.19	0.63	0.20	0.50	0.67	0.64	0.74	0.89	0.24	0.21	0.18	0.43	0.70	0.12	0.44	0.61	0.53	0.53	0.58
95	0	0	0	0	0	0	0	0	0	0	0.10	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	1	1	1	1	1	0.82	0.60	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
105	0	0	0	0	0	0	0	0	0	0	0.10	0.05	0	0	0	0	0.06	0	0	0	0	0	0	0	0	0	0
110	0.33	0.33	0.22	0.24	0.39	0.94	0.90	0.53	0.69	0.81	0.50	0.80	0.44	0.82	0.58	0.56	0.24	0.84	0.82	0.79	0.55	0.59	0.56	0.56	0.47	0.53	0.47
120	0	0	0	0	0	0	0	0	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nb. of alleles	13	15	11	14	11	12	10	12	16	14	17	14	14	14	12	14	12	11	14	16	15	12	15	15	16	12	14

Table 3 continued.

	Sites										(B)	Zones				Regions		
	78	81	86	89	92	97	102	107	114	117		I	II	III	IV	N	W	E
Locus																		
Mdh																		
100	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1
121	0	0.21	0	0.07	0	0.07	0.13	0	0	0.22		0.02	0.08	0.18	0.05	0.02	0.11	0.08
Pgd																		
80	0	0	0	0	0	0.07	0	0	0	0		0	0.05	0	0.04	0	0.06	0.03
94	0	0	0	0	0	0	0	0	0	0		0	0.006	0	0	0	0.01	0
100	1	1	1	1	1	1	1	1	1	1		1	0.99	1	1	1	0.99	1
106	0	0	0	0	0	0	0	0	0	0		0	0.006	0	0	0	0.01	0
Pgm																		
92	0	0	0	0	0	0	0	0	0	0		0.04	0.01	0	0	0.04	0.02	0.003
96	0.88	0.14	0.43	0.87	0.30	0.43	0.25	0.42	0.67	0.67		0.64	0.46	0.43	0.51	0.64	0.48	0.46
100	1	1	1	1	1	1	1	1	1	1		0.86	0.99	1	1	0.86	0.99	0.99
104	0.12	0.29	0.93	0.87	0.70	1	0.88	0.25	0.28	0.44		0.51	0.51	0.33	0.58	0.51	0.58	0.46
108	0	0	0.43	0.07	0.40	0.43	0.19	0.08	0	0		0	0.04	0.13	0.19	0	0.03	0.11
Idh																		
76	0	0	0	0	0	0.29	0	0	0.28	0		0	0.02	0.05	0.04	0	0.006	0.04
84	0.06	0	0	0	0.05	0.36	0	0.33	0.33	0.17		0.32	0.16	0.12	0.11	0.32	0.24	0.10
92	0.12	0	0	0	0.05	0	0	0	0.06	0.17		0.08	0.11	0.01	0.08	0.08	0.03	0.11
100	0.88	1	1	1	0.95	1	1	1	0.94	0.83		0.92	0.89	0.99	0.92	0.92	0.97	0.89
108	0.12	0	0	0	0.05	0	0	0	0.06	0.17		0.08	0.11	0.01	0.08	0.08	0.03	0.11
116	0	0	0	0	0	0	0	0	0	0		0.01	0.01	0.02	0	0.01	0.03	0
Gpi																		
40	0	0	0	0	0	0	0	0	0	0		0	0.006	0	0	0	0	0.005
60	0	0	0	0	0	0	0	0	0	0		0	0.006	0	0	0	0	0.005
80	0.41	0.07	0.57	0.87	0.15	0.07	0	0.50	0.44	0		0.04	0.09	0.28	0,11	0.04	0.12	0.20
90	0.53	0.14	1	0.93	0.90	0.57	0.13	0.75	0.50	0.44		0.34	0.41	0.51	0,28	0.34	0.52	0.47
95	0	0	0	0	0	0	0	0	0	0		0	0.008	0	0	0	0.02	0
100	1	1	1	1	1	1	1	1	1	1		1	0.97	1	0,42	1	1	0.97
105	0	0	0	0	0	0	0	0	0	0		0	0.008	0.01	0	0	0.02	0
110	0.59	0.21	0.21	0.80	0.75	0.21	0.69	0.17	0.56	0.56		0.34	0.68	0.46	0,19	0.34	0.60	0.58
120	0	0	0	0	0	0	0	0	0	0		0	0.006	0	0	0	0.007	0
Nb. of alleles	13	11	11	12	14	15	11	12	14	13		16	26	18	17	16	24	20

Table 4. The distribution of 146 genotypes over 4 vegetation zones or 3 regions. See 'Material and Methods' for explanation of the zones and regions.

Genotypes present	Zone	I	II	III	IV	Region	N	W	E
1 site		13	48	11	12		13	43	28
1 zone/region predominantly		2	7	1	0		2	14	2
1 zone/region		3	5	0	0		3	8	7
all zones/regions	44					26			
Mean per zone/region		3.0	3.3	2.0	1.7		3.0	3.0	4.1

A and Ho related to geographical patterns

In Table 5a the mean number of alleles (A) and the mean observed heterozygosity across the loci (Ho) are given for the 37 sites, grouped into four vegetation zones or three regions (Table 5b). The mean number of alleles and the observed heterozygosity did not differ much among the zones or regions; they were slightly lower in zone I (or in the northern region) than in the other zones or regions. Site 19 and 21, the sites with the diploid populations, were among the sites with the lowest mean number of alleles and observed heterozygosity. Only site 48 (only hexaploid *P. setosum* samples) and 81 (mainly tetraploid *P. subangustum* samples) had an even lower Ho than the diploids.

Correspondence analysis

The maximum number of axes to be obtained per analysis equals the number of variables or categories minus one. The scatterplots shown in the figures 4 to 8, are all made with the first two axes (factors). Up to four axes (Table 7) have been taken into account for the determination of the factors explaining the variation, but the scatterplots with factors 3 and 4 are not shown. Absolute contributions to the different axes of the variables were assessed globally by the formula: (1000/nb of variables) x 2.

Nine rare alleles out of the 26 alleles were left out of the analyses because they prevented scattering of the other variables. Those alleles are: *Pgd* 94 and 106, *Pgm* 92, *Idh* 116, and *Gpi* 40, 60, 95, 105, and 120. For the categories species and ploidy level, certain proportions of alleles were set at 0 (Table 5), because the low number of

Table 5a. Mean number of alleles (A) and mean observed heterozygosity (Ho) for the 37 sites.

Site	Zone	Region	Number of Samples	% different Genotypes	A	Ho	Species present*
1	I	N	15	67	1.4	0.31	P,O
2	I	N	18	61	1.4	0.37	P,O,(S)
3	I	N	18	44	1.4	0.37	P,O,S
9	I	N	17	71	1.4	0.34	P,O,S
13	I	N	23	70	1.5	0.35	P,O,S
19	II	W	17	41	1.3	0.25	O,P,S
21	II	W	20	50	1.2	0.20	O,(S)
23	I	N	15	93	1.5	0.35	O,S,P,H
26	II	E	16	75	1.6	0.40	O,S,P
27	II	E	16	63	1.7	0.49	O,S,(P)
29	II	E	20	60	1.6	0.41	O,S,P
30	II	E	20	75	1.7	0.47	O,P,S,H
31	II	E	18	72	1.5	0.38	O,S,P
37	II	E	11	64	1.6	0.47	O,H,(S),(P)
38	II	E	19	53	1.6	0.46	E,O,S
43	III	E	18	61	1.7	0.49	O,E,S
48	III	E	17	53	1.2	0.16	E
56	II	W	19	58	1.4	0.33	P,S,O
58	II	W	21	57	1.3	0.31	O,P,S,(H)
59	II	W	15	93	1.6	0.41	P,O,E,S
61	II	W	20	80	1.6	0.51	S,H,O,P
62	II	W	17	47	1.5	0.33	E,S,(O)
66	II	W	16	75	1.6	0.46	S,O,(H)
67	II	W	18	72	1.6	0.46	O,S,E,H
68	II	W	17	65	1.6	0.47	O,S,H,(E)
72	III	W	17	41	1.5	0.34	E,S,H
75	IV	W	19	47	1.5	0.52	S,E,O
78	IV	W	17	47	1.5	0.35	S,(H)
81	IV	W	14	36	1.2	0.19	S,(O),(H)
86	IV	W	14	36	1.7	0.39	E,S,(O)
89	IV	W	15	27	1.9	0.37	S,(H)
92	IV	W	20	60	1.7	0.40	E,P,S,(O)
97	IV	W	14	57	1.7	0.50	S,E,(H)
102	III	W	16	50	1.5	0.36	O,E,S,(H)
107	III	W	12	50	1.6	0.37	S,E,A,(O)
114	III	W	18	61	1.5	0.49	O,P,S,H,(E)
117	II	W	18	39	1.5	0.47	O,H

* P = *P. pedicellatum*, O = *P. polystachion*, S = *P. subangustum*, H = *P. hordeoides*, E = *P. setosum*, and A = *P. atrichum*

The letters between brackets indicate species that are 1-2 times present in the sample.

Table 5b. Mean number of alleles (A) and mean observed heterozygosity (Ho) for the 4 vegetation zones and 3 regions.
See 'Material and Methods' for explanation of the different zones and regions.

Zone	A	Ho
I	1.4	0.35
II	1.5	0.40
III	1.5	0.37
IV	1.6	0.39

Region	A	Ho
N	1.4	0.35
E	1.6	0.41
W	1.5	0.35

samples in some groups restricted the distinction level. Significant differences within categories are all relative, because the 17 alleles taken into account occur in different quantities in most groups of each of the categories.

Correspondence analysis of the species

The rare species *P. atrichum* was left out of the analysis. The proportion of each of the species per allele is given in Table 6. Table 7 presents the alleles and species contributing to the different axes, as well as the variation explained by each axis.

P. setosum has significantly more *Pgm* 108 (χ^2 = 43.52; d.f. = 4; P<0.01) and significantly less *Gpi* 80 (χ^2 = 46.43; d.f. = 4; P<0.01) than the other species. *P. pedicellatum* has significantly less *Mdh* 121 (χ^2 = 28.99; d.f. = 4; P<0.01) than *P. hordeoides*, *P. polystachion* and *P. subangustum*. *P. hordeoides* has significantly more *Gpi* 110 (χ^2 = 6.87; d.f. = 2; P<0.05) than *P. subangustum* and *P. setosum*. *P. subangustum* has significantly more *Gpi* 80 (χ^2 = 22.23; d.f. = 4; P<0.01) than *P. polystachion*, *P. pedicellatum*, and *P. setosum*, and significantly less *Gpi* 110 (χ^2 = 9.94; d.f. = 1; P<0.01) than *P. polystachion*. Only *P. polystachion* and *P. hordeoides* did not show significantly differences for any of the alleles. The factorial scatterplot with factors 1 and 2 is given in Fig. 4. All species are largely overlapping, but *P. pedicellatum* has a more restricted distribution than the other species. Correspondence analysis of the different chromosomal taxa did not result in different patterns, and are not shown.

Correspondence analysis of the ploidy levels

Three axes could maximally be calculated, the total variation explained by them was 100%. The proportion of each of the ploidy levels per allele is given in Table 6. Table 7 presents the alleles and ploidy levels contributing to the different axes, as well as the variation explained by each axis.

Table 6. Average number of samples per category and per allel, used for the different correspondence analyses. The number between brackets indicates the real number, which has been set at 0 for the analysis.

Category	*M100	M121	D80	D10	P96	P100	P104	P108	I76	I84	I92	I100	I108	G80	G90	G100	G110
Species																	
E	1	0 (0.02)	0 (0.01)	1	0.57	1	0.68	0.30	0 (0.02)	0.05	0.06	0.94	0.06	0 (0.02)	0.57	1	0.43
H	1	0.21	0.07	1	0.29	0.95	0.55	0 (0.02)	0	0.19	0.10	0.90	0.10	0.21	0.48	1	0.71
O	1	0.11	0 (0.02)	0.99	0.46	0.99	0.45	0.05	0.04	0.16	0.13	0.87	0.13	0.09	0.42	0.95	0.69
P	1	0 (0.02)	0.05	1	0.62	0.92	0.51	0	0 (0.02)	0.25	0.04	0.96	0.04	0.08	0.36	1	0.58
S	1	0.1	0.04	1	0.48	0.96	0.43	0.04	0.03	0.18	0.09	0.91	0.09	0.31	0.49	0.99	0.47
Ploidy level																	
2x	1	0	0.10	1	0.14	0.93	0.21	0	0	0 (0.03)	0	1	0	0	0	0.62	0.90
4x	1	0.08	0 (0.03)	1	0.50	0.96	0.45	0 (0.03)	0 (0.03)	0.17	0.11	0.89	0.11	0.19	0.47	1	0.53
5x	1	0.06	0	1	0.56	0.94	0.56	0.25	0.13	0	0	1	0	0	0.44	1	0.63
6x	1	0.09	0 (0.02)	0.99	0.57	1	0.71	0.19	0 (0.00)	0.20	0 (0.03)	0.97	0 (0.03)	0 (0.03)	0.48	1	0.55
Zone																	
I	1	0.02	0	1	0.64	0.85	0.51	0	0	0.33	0.08	0.92	0.08	0.05	0.34	0.99	0.34
II	1	0.08	0.05	0.99	0.47	0.99	0.52	0.04	0.03	0.15	0.11	0.89	0.11	0.09	0.40	0.97	0.68
III	1	0.19	0	1	0.44	1	0.33	0.12	0.05	0.11	0.01	0.99	0.01	0.28	0.50	1	0.47
IV	1	0.04	0.04	1	0.51	1	0.56	0.19	0.04	0.12	0.10	0.90	0.10	0.30	0.67	1	0.49
Region																	
N	1	0.02	0	1	0.64	0.84	0.51	0	0	0.33	0.08	0.92	0.08	0.05	0.34	0.99	0.34
E	1	0.12	0.06	0.99	0.50	0.99	0.58	0.03	0.01	0.22	0.03	0.97	0.03	0.12	0.52	1	0.59
W	1	0.08	0.03	1	0.46	0.99	0.45	0.11	0.04	0.10	0.12	0.89	0.12	0.19	0.46	0.97	0.60

*M = *Mdh*, D = *Pgd*, P = *Pgm*, I = *Idh*, and G = *Gpi*

Table 7. Maximum number of axes per analysis, the percentage of variation explained per axis, and the categories and alleles with high factor loadings explaining the axes.

Axis	Variation (%)	Category	Allele*
		Species	
1	61.9	E, H	P108, G80, M121
2	19.1	P, H	M121, P108, I84
3	9.9	S, H	G80, G110
4	9.1	O, H	I76, D80
Total	100		
		Ploidy level	
1	51.4	2x, 4x	D80, G110, G90
2	37.9	4x, 5x	P108, G80, I92/I108
3	10.7	6x, 5x	I76, I84
Total	100		
		Vegetation zone	
1	60.4	I, III	G80, I84, P108
2	24.1	III, IV	M121, D80, I92/I108
3	15.5	II, IV	G110, M121, P108, G80
Total	100		
		Vertical region	
1	72.5	N, W	I84, P108, G80
2	27.5	E, (N, W)	D80, M121, I92/I108
Total	100		

* M=*Mdh*, D=*Pgd*, P=*Pgm*, I=*Idh*, and G=*Gpi*.

The diploids have significantly more *Gpi* 110 (χ^2 = 17.34; d.f. = 2; P<0.01) and *Pgd* 80 (χ^2 = 11.52; d.f. = 2; P<0.01) than the tetraploids. The tetraploids had significantly more *Gpi* 90 (χ^2 = 18.01; d.f. = 2; P<0.01) than the diploids, significantly more *Ihd* 92/*Idh* 108 (χ^2 = 11.49; d.f. = 2; P<0.01) and *Gpi* 80 (χ^2 = 8.84; d.f. = 2; P<0.05) than the pentaploids. The pentaploids had significantly more *Pgm* 108 (χ^2 = 10.61; d.f. = 2; P<0.01) than the tetraploids, and significantly more *Idh* 76 (χ^2 = 15.33;

d.f. = 2; P<0.01) than the tetra- and hexaploids. A scatterplot with factors 1 and 2 is given in Figure 5. Many of the 635 samples are not visualized because they overlap with the samples shown in the scatterplot. The tetraploids include the other ploidy levels for the largest part. The diploids and pentaploids show only a small overlap in the upper part of the figure.

Correspondence analysis of the geographical zones or regions

The proportion of each of the zones per allele is given in Table 6. Table 7 presents the alleles and zones contributing to the three axes, as well as the variation explained by each axis.

Zone I has significantly more *Idh* 84 (χ^2 = 19.78; d.f. = 3; P<0.01) than Zones II, III and IV. Zone II has significantly more *Mdh* 121 (χ^2 = 8.66; d.f. = 3; P<0.05) and *Gpi* 110 (χ^2 = 7.12; d.f. = 3; P<0.05) than Zones I, III and IV, and significantly more *Idh* 92/*Idh* 108 (χ^2 = 12.59; d.f. = 3; P<0.01) than Zone III. Zone III has significantly more *Pgm* 108 (χ^2 = 12.81; d.f. = 3; P<0.01) and *Gpi* 80 (χ^2 = 14.19; d.f. = 3; P<0.01) than Zones I and II, and significantly more *Mdh* 121 (χ^2 = 11.85; d.f. = 3; P<0.01) than Zones I, II and IV. Finally, Zone IV has significantly more *Idh* 92/*Idh* 108 (χ^2 = 8.13; d.f. = 3; P<0.05) than Zone III, and significantly more *Pgm* 108 (χ^2 = 19.38; d.f. = 3; P<0.01) and *Gpi* 80 (χ^2 = 16.68; d.f. = 3; P<0.01) than Zones I and II. The scatterplot with factors 1 and 2 is given in Fig. 6. The sites from vegetation zone I form a separate group from the sites of the other zones. Most sites of zone IV are overlapping with those from zone III, with the exception of site 75, which is different from all the other sites.

The proportion of each of the three regions per allele is given in Table 6. Table 7 presents the alleles and regions contributing to both axes, as well as the variation explained by each axis.

The northern (N) dry region (southern Niger and most of Burkina Faso) has significantly more *Idh* 84 (χ^2 = 21.49; d.f. = 2; P<0.01) than the western (W) region (Ivory Coast plus diploids). The western region has significantly more *Pgm* 108 (χ^2 = 13.14; d.f. = 2; P<0.01) than the northern and eastern region, significantly more *Gpi* 80 (χ^2 = 7.75; d.f. = 2; P<0.05) than the northern region, and significantly more *Idh* 92/*Idh* 108 (χ^2 = 8.32; d.f. = 2; P<0.05) than the eastern (E) region (Benin). The eastern region has significantly more *Mdh* 121 (χ^2 = 6.21; d.f. = 2; P<0.05) and *Pgd* 80 (χ^2 = 6.39; d.f. = 2; P<0.05) than the northern region, and significantly more *Idh* 84 (χ^2 = 10.45; d.f. = 2; P<0.01) than the western region. The factorial scatterplot is given in Fig. 7. The sites from the northern region are completely separated from the other two regions, confirming the results from the analysis of the four vegetation zones, while the sites of the eastern region largely overlap with those from the western region.

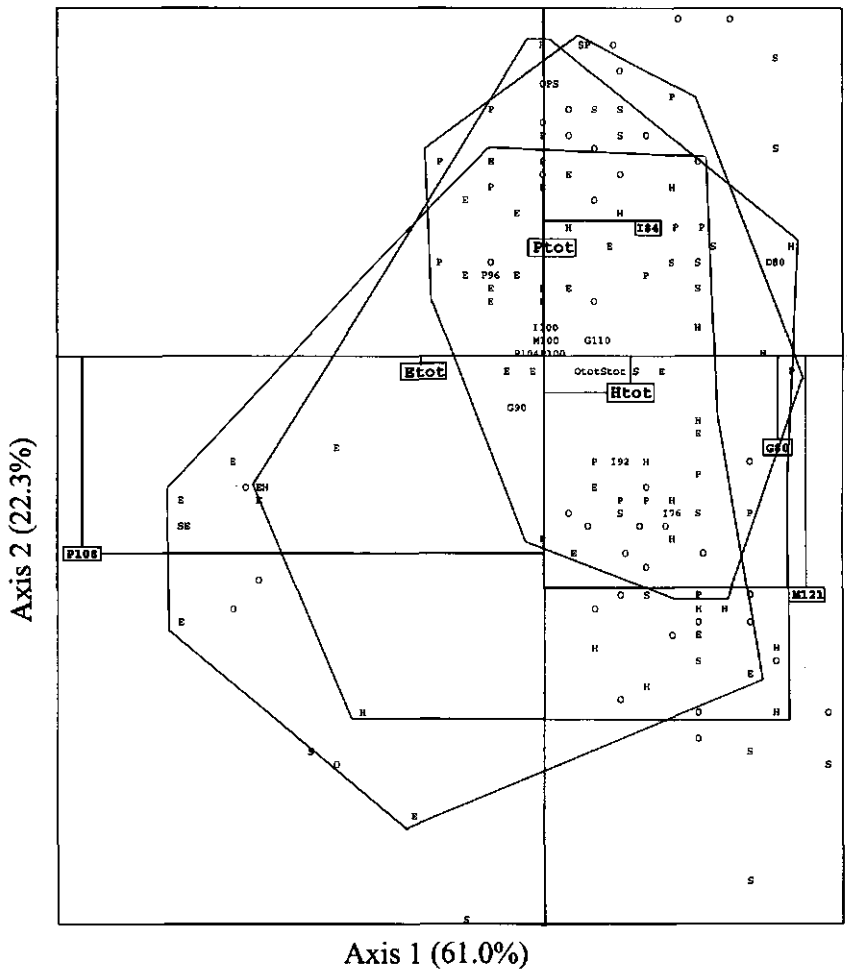


Figure 4. Scatterplot of axes 1 and 2 from the correspondence analysis of the species. The boxed units are the factors with high loadings on these axes: Ptot = *P. pedicellatum*, Htot = *P. hordeoides*, and Etot = *P. setosum* for the species; P108 = *Pgm* 108, I84 = *Idh* 84, G80 = *Gpi* 80 and M121 = *Mdh* 121 for the alleles. A line connects each factor and the axis explained by it. The (visible) individual samples of each species, P = *P. pedicellatum*, O = *P. polystachion*, S = *P. subangustum*, E = *P. setosum*, and H = *P. hordeoides*) are plotted, and outlines indicate the distribution of the species with high factor loadings over the plot.

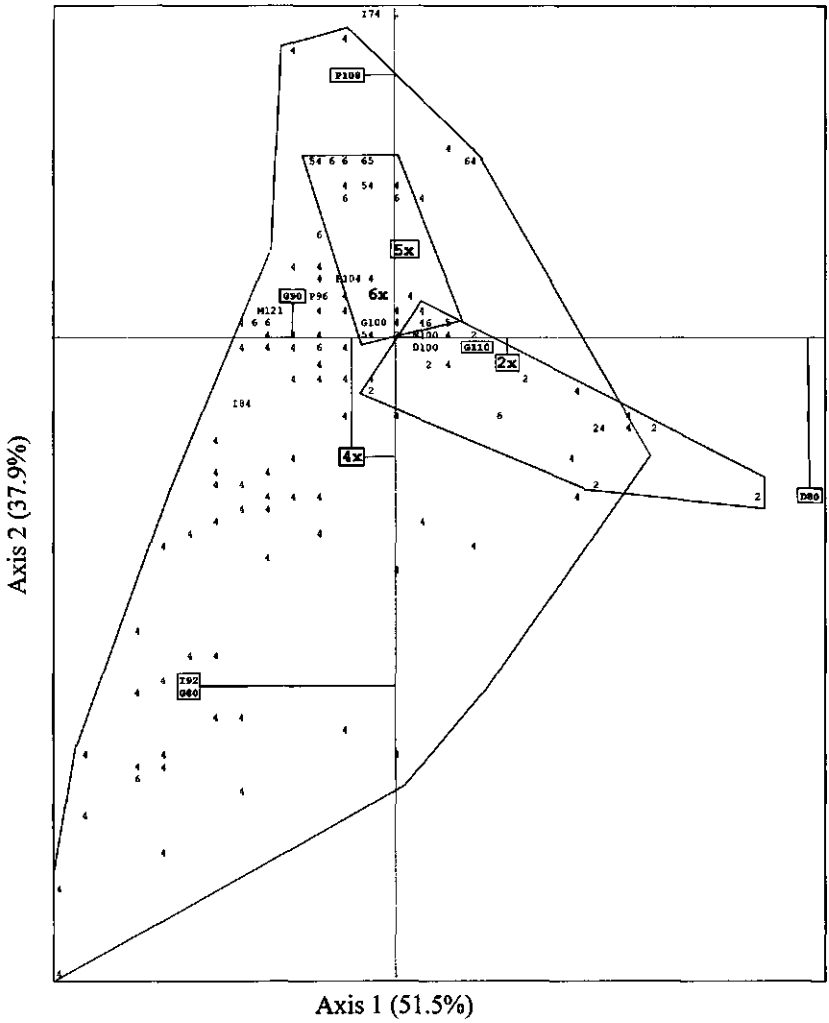


Figure 5. Scatterplot of axes 1 and 2 from the correspondence analysis of the ploidy levels. The boxed units are the factors with high loadings on these axes: 2x = diploids, 4x = tetraploids, and 5x = pentaploids; D80 = *Pgd* 80, P108 = *Pgm* 108, I92 = *Idh* 92, and G80, G90 and G110 = *Gpi* 80, 90 and 110 for the alleles. A line connects each factor and the axis explained by it. The (visible) individual samples of each ploidy level, 2 = diploids, 4 = tetraploid, 5 = pentaploids, and 6 = hexaploids are plotted, and outlines indicate the distribution of the ploidy levels with high factor loadings over the plot.

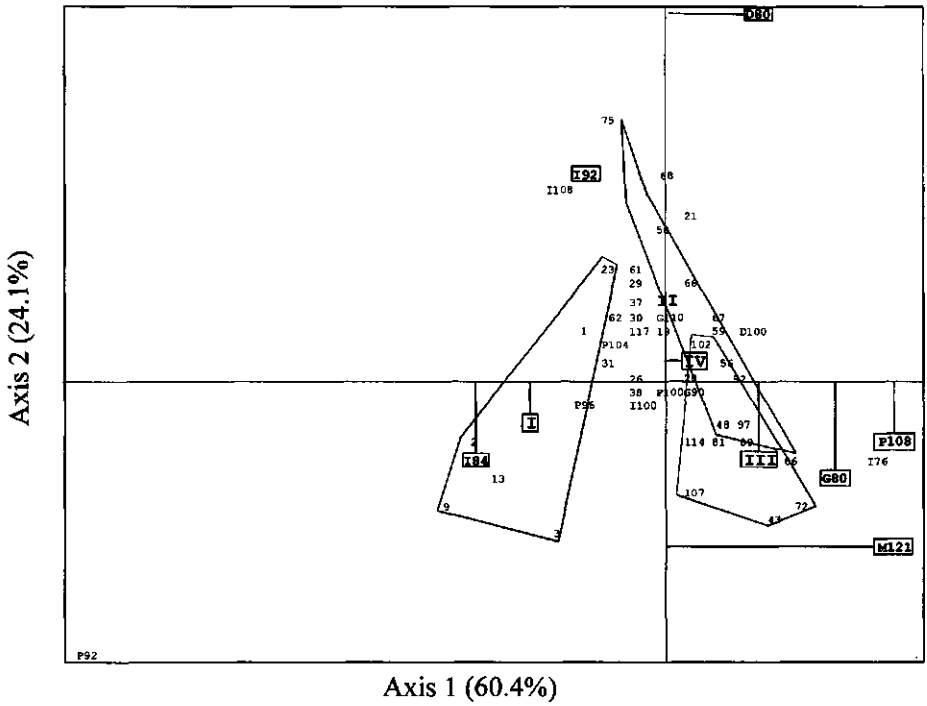


Figure 6. Scatterplot of axes 1 and 2 from the correspondence analysis of the four vegetation zones. The boxed units are the factors with high loadings on these axes: I = zone I, III = zone III, and IV = zone IV; D80 = *Pgd* 80, P108 = *Pgm* 108, I84 and I92 = *Idh* 84 and 92, M121 = *Mdh* 121, and G80 = *Gpi* 80 for the alleles. A line connects each factor and the axis explained by it. The sites belongin to each zone are plotted, and outlines indicate the distribution of the zones with high factor loadings over the plot.

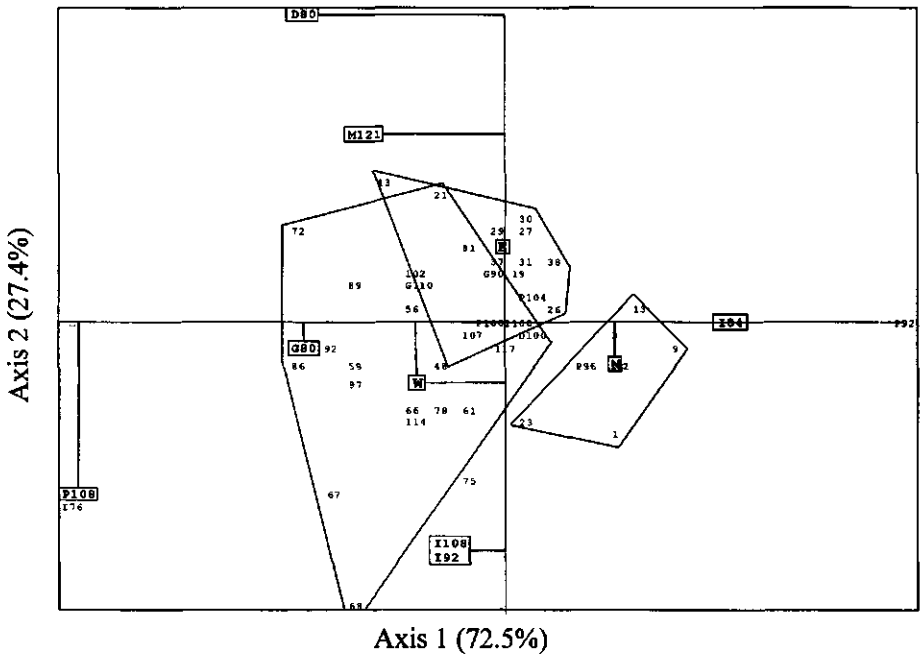


Figure 7. Scatterplot of both axes from the correspondence analysis of the three regions. The boxed units are the factors with high loadings on these axes: E = eastern region, W = western region, and N = northern region; D80 = *Pgd* 80, P108 = *Pgm* 108, I84 and I92 = *Idh* 84 and 92, M121 = *Mdh* 121, and G80 = *Gpi* 80 for the alleles. A line connects each factor and the axis explained by it. The sites belonging to each region are plotted, and outlines indicate the distribution of the regions over the plot.

Discussion

The results of the isozyme and correspondence analysis can be summarized as follows:
(1) In 635 genitors 146 different 5-locus genotypes were found, through combination of 26 alleles.
(2) Different morphological species and chromosomal taxa, in many different combinations, shared 42.5% of these genotypes, in 86.8% of the genitors.
(3) In *P. polystachion* all 26 alleles were found, but when the 6 rare alleles were removed, most differences with the other species disappeared, showing that the species belong to the same gene pool.
(4) Most alleles were found in the tetraploids, and least in the di- and pentaploids. In *Mdh*, *Pgd* and *Idh* two alleles represented a heterozygote; in *Pgm* and *Gpi* there could be up to four alleles in heterozygotous polyploids. The diploids had significantly less

(two-allele) heterozygotes than the tetra- and hexaploids for *Idh*, *Pgm* and *Gpi*. Significantly more three-allele heterozygotes were found in penta- and hexaploids, compared to tetraploids. Also, significantly more four-allele heterozygotes were found in hexaploids compared to tetra- and pentaploids.
(5) The mean number of alleles (A) over 5 loci was 1.5. The mean observed heterozygosity (Ho) of the samples in this study was 0.38. A and Ho are significantly lower in the diploids (A = 1.2 and Ho = 0.15 to 0.19). For the diploids (the samples from *P. polystachion* and *P. subangustum* taken together) the expected heterozygosity (He) was not significantly different from Ho, and the hypothesis that the diploids are reproducing randomly could not be rejected. Small, non-significant differences exist between the A and Ho of the species and the geographical zones and regions.
(6) Correspondence analysis showed that the distribution of different proportions of some of the common alleles was significantly different between the species (without *P. atrichum*), between most of the chromosomal taxa (without *P. atrichum*, pentaploid *P. setosum*, penta- and hexaploid *P. hordeoides*, and diploid *P. subangustum*), between all the ploidy levels, and between all four vegetation zones or the three regions.

Electrophoretical analysis of 635 samples from *Pennisetum* section *Brevivalvula* showed that although the samples seem to share the same gene pool, different patterns exist between the morphological species, the ploidy levels, and that a geographical effect is also present. The lack of clear genetic difference confirms the lack of (strong) morphological divergence, found in Chapter 4. Although a restricted allozyme polymorphism was detected in the section by the 5 enzymes used, and probably more differences will be found with more enzymes, part of the genitors from different morphological species could be really similar. A preliminary study of chloroplast-DNA in the section revealed three main groups: (1) part of the diploids (from *P. polystachion* and *P. subangustum*), (2) *P. pedicellatum* (the northern species), (3) part of the diploids and samples from the other species (pers comm. Renno). Extensive gene flow caused by the diploids found in this study, and by residual sexuality of the apomictic polyploids (see Chapter 3), through the model of diploid-tetraploid-haploid cycles, described by De Wet (1968), could explain this morphological and genetical similarity.

Still, morphological differentiation exists, and is partly confirmed genetically. Polyploid genitors from identical chromosomal taxa, originating from the same site and having the same genotype, are morphologically similar as well (hairiness and inflorescence color) and probably belong to the same clone. This similarity is often not present between genitors from the same chromosomal taxon, having the same genotype but originating from different sites. In those cases different parents are probably involved.

Most analyzed sites in this study have a high genetic diversity. This high genetic diversity could be related to the selection of the morphologically most polymorphic sites for analysis. Sites with relatively low morphological polymorphism sometimes have low genetical polymorphism. This is observed Table 5a) in site 48 in the south of

Benin with predominantly hexaploid *P. setosum*, and in site 81 in the south of Ivory Coast, with predominantly tetraploid *P. subangustum*, growing along a forest road that was constructed in the 1950's. This pattern could be explained by attributing it to recent colonization, which is probably effected by a few genetically identical clones at a time, leaving less space for morphological polymorphism as well. *P. setosum* is one of the species where no variable progeny has been found (Chapter 3), an indicating that it could be among the most apomictic species of the complex.

In areas where sexuals and apomicts occur simultaneously, like the Banfora area in Burkina Faso, hybridization events are turning out a multitude of morphologically and genetically distinct biotypes, that can maintain a certain level of gene exchange. It is less likely that stable, morphological recognizable entities can develop in areas like this.

Hamrick & al. (1979) and Hamrick & Godt (1990) observed allozyme diversity in 473 plant species. They found that species reproducing sexually and asexually had a mean number of alleles (A) of 1.69, and an observed heterozygosity level (Ho) of 0.14. These numbers are close to A = 1.5, and Ho = 0.15-0.19 found in this study. The mean Ho of the polyploids in this study was significantly higher (Ho = 0.38) than those of the diploids. These results are contradicting those of Assienan & Noirot (1995), who studied diploids and polyploids in the agamic complex of *Panicum maximum* and related species. They found the heterozygosity level within apomicts as high as that of sexuals. Despite the existence of three to four alleles per locus, only simplex or duplex structures were observed. In *Pennisetum* section *Brevivalvula* the heterozygosity level in the polyploids was significantly higher than the diploids, because tri- and quadruplex structures were present. These multi-allelic heterozygotes were mostly not caused by rare alleles, the six rare alleles in this study were responsible for only 4 genotypes. High heterozygosity levels in obligate apomicts are normally caused by fixation of functional, possible harmful, mutations, and non-functional, potentially advantageous mutations. Even a limited amount of outcrossing acts as a filter that diminishes the accumulation rate of these deleterious mutations. This effect has an extra dimension in predominantly annual apomictic complexes like *Pennisetum* section *Brevivalvula*, where each year a new generation has to be produced to guarantee the survival of the group.

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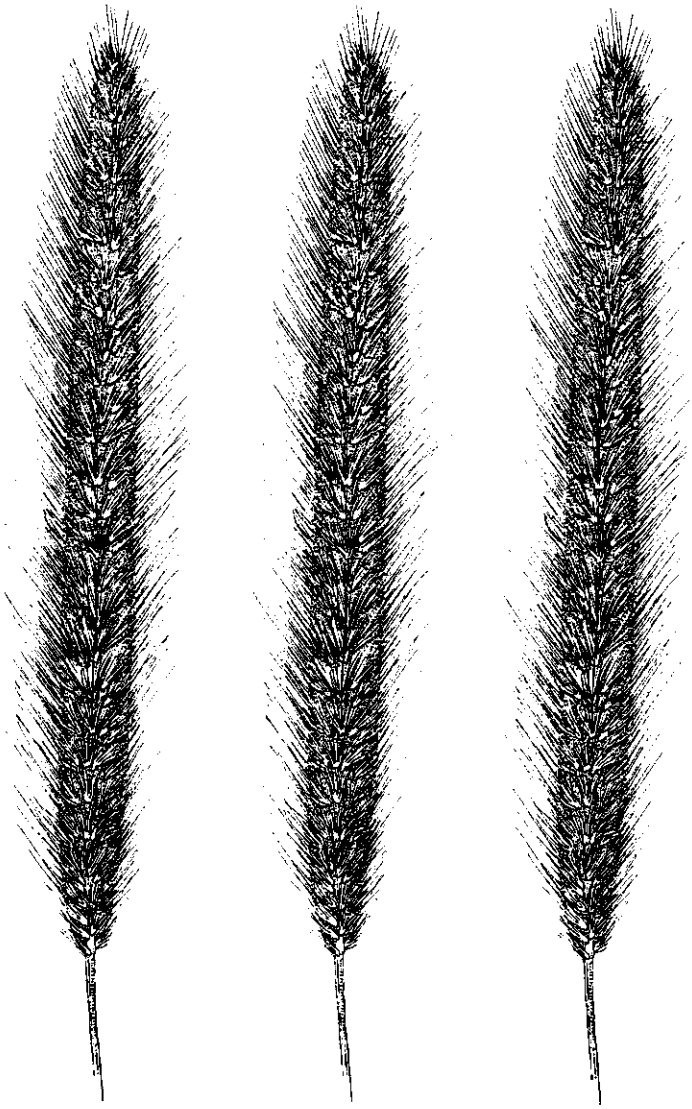

Pennisetum setosum

Chapter 6

GENERAL DISCUSSION

In this chapter the principal results from this study of the agamic species complex *Pennisetum* section *Brevivalvula* are discussed. Some perspectives for future study and possible uses of *Pennisetum* section *Brevivalvula* are highlighted at the end.

Polyploidy, apomixis and life cycle

Polyploidy is normally caused by hybridizations between closely related taxa at species level or at a lower level. Implications of these hybridizations are often associated with physiological or ecological tendencies: (1) increase in production of enzymes and chemicals, changing the physiology of a plant, (2) (partial) sterility, leading to a restricted competitive power compared to the diploid parents, (3) buffer against the effects of (positive and negative) mutations, (4) vigorous plants (larger seeds, higher plants, bigger inflorescences), (5) tendency to a perennial life cycle, (6) tendency to vegetative propagation, (7) tendency to an increased stress tolerance, due to higher enzyme activity and greater homeostasis, which increases the colonizing capacity (Jackson, 1982; Asker & Jerling, 1992).

Implications of apomixis are: (1) production of large numbers of genetically identical plants, reducing the risk of a genotype becoming extinct, (2) long distance dispersal by seeds (compared to short distance vegetative propagation), (3) fixation of heterozygosity (and of heterosis), thus prevention of (sub)lethal homozygotes causing inbreeding depression, or worse: elimination of the genotype (Asker & Jerling, 1992).

The combination of apomixis and polyploidy can lead to the restoration of fertility in sexually sterile individuals. The advantage of a perennial life cycle in such a system is that a sterile perennial, provided it is vigorous, may live for a long time; when combined with vegetative propagation, it can even form a large clone. Many largely sterile species of hybrid origin are saved by the adoption of a vegetative or apomictic mode of reproduction or both. Agamic complexes are believed to be mainly of hybrid origin, because high levels of heterozygosity are often found. Polyploids can easily be formed through residual sexuality in the agamic complex, and stabilize this heterozygosity.

Asexual seed formation, or agamospermy, in the *Panicoideae* is effected through parthenogenetic development of egg cells in unreduced embryo sacs. The embryo sacs are formed from somatic cells in the ovule (apospory), and are four-nucleate and

monopolar. Fertilization of the central nucleus is necessary for endosperm development (pseudogamy) and thus with seed formation, and pseudogamy is often related to facultative apomixis. Facultative apomicts produce (mostly) good pollen, and also sexual and apomictic embryo sacs. The frequency of these different types of embryo sacs depends on the degree of apomictic production in the parent plant, which in its turn is depending often on environmental conditions. This combined reproduction results in mixed progeny of maternal and aberrant types, although most offspring is clonally formed.

The agamic complex of *Pennisetum* section *Brevivalvula* exists for a larger part of tetraploid apomictic annual species, although penta- and hexaploid cytotypes are also found. These species are *P. pedicellatum*, *P. polystachion*, *P. hordeoides* and *P. subangustum*. Two perennial taxa can be distinguished as well: *P. setosum* is mainly hexaploid, with some tetra- and pentaploid cytotypes, and *P. atrichum* is tetraploid. Low levels of variation in genotypes have been shown to exist in the progeny of several of the taxa, in all ploidy levels. This genotypic variation can be explained by the fertilization of sexual gametes (facultative apomixis), or by non-sexual, rare genetic mutations.

Sexual reproducing diploids are found in *P. polystachion* and *P. subangustum*, in a relatively small area near Banfora, in Burkina Faso. From the literature a single reference of diploid *P. hordeoides* is known. Unpublished data from Senegal confirm the existence of other diploid populations in this taxon.

Annual apomicts are restricted in the possibilities of perpetuating genotypes with a disturbed meiosis, because if viable seeds are not produced at the end of the growth season, the genotype will cease to exist. The perennials in the section do not have this acute problem, because they can persist several growth seasons. They do not possess of the additional advantage of vegetative reproduction, which many perennials do have. The formation of secondary roots at the lower nodes is a feature many grasses have, and it is also present in section *Brevivalvula*. In time it could become adapted as a means of vegetative propagation in perennials. Some of the annuals from sub-humid zones, including the diploids, show a tendency to prolong their life cycle, and become intermediates between annuals and perennials, in the sense that they can persist for some time after flowering and form some new green shoots, if the rains allow this to happen. Most annuals will die as soon as they have finished flowering. The perennials can survive a relatively short period of drought, up to about 4 months.

Genotypic (dis)similarity in *Pennisetum* section *Brevivalvula*

The species of *Pennisetum* section *Brevivalvula* in West Africa are showing their largest morphological overlap in the second vegetation zone. This zone is dynamic because it is the buffer zone between the dry sahelian zone and the humid rain forest

area. Areas of relief, providing many possibilities for niche differentiation, characterize the sampled regions in this zone. All ploidy levels and most species (except the rare *P. atrichum*) are found in this zone. Aneuploids were not observed in the samples, possibly because the flow cytometry method used did not allow this, or because aneuploids cannot survive for a long time in a population. Diploid sexuals, from *P. polystachion* and *P. subangustum*, were sampled at two sites in this zone only, between Banfora and the border with Mali. One site showed a mixture of diploids and polyploids of *P. pedicellatum*, and at the other site, closer to the Mali border, only diploids were collected. No more sites were sampled after this last one. The absence of polyploids in the last site could be an indication that it was more in the center of the diploid population. Both sites where diploids were found were almost 130 km apart. It is difficult to estimate the area the diploid population covered, but it could be considerable. At the same sites where the diploid *P. polystachion* and *P. subangustum* were found, tetraploid cytotypes of the same species with 5-locus genotypes identical to the diploids were collected. Only one of the other species, *P. pedicellatum*, was also sampled near the diploids, with all of its known cytotypes, tetra-, penta-, and hexaploid. One putative hybrid between *P. pedicellatum* and *P. polystachion*, *P. pedicellatum* ssp. *unispiculum*, was collected here as well. Some of the *P. pedicellatum* samples had the same 5-locus genotype as the diploids. The remaining three species were not sampled in the direct vicinity of the diploids, although they could well exist close by. This cytological variation in the *P. pedicellatum* and *P. polystachion* samples are an indication that the diploid sexuals are causing genetic variation in the complex. The morphological and genetic variation in this whole region is higher than in the other regions, which strengthens this assumption.

The 5-locus genotypes occur in different patterns over the morphological species. Five groups are distinguished: (1) 5-locus genotypes that are found in only one individual plant, (2) 5-locus genotypes that are found in several plants of the same species at the same site, (3) 5-locus genotypes that are found in several plants of the same species at different sites, (4) 5-locus genotypes that are found in different species at the same site, or (5) 5-locus genotypes that are found in different species at different sites. In case 2 and 3 different cytotypes of the same species can be involved. When identical genotypes were found in the same species at the same sites, the inflorescence color was often the same as well, but this was mostly not the case with species from different sites. In the first example the samples could be clonally formed, whereas in the second example they are probably not. In case 4 and 5, different species are sharing the same 5-locus genotype. Mostly the number of samples involved per 5-locus genotype in these cases is low, two or three samples is most common. Some 5-locus genotypes are found in many samples though, in which all species and cytotypes are present.

The genotypic differences found between species could well be as large as the genotypic differences within a species. Apart from indicating that the taxa in *Pennisetum* section *Brevivalvula* form indeed a species complex, probably sharing the

same gene pool, the results neither really contradict nor confirm the morphological and ecological groups observed.

Morphological and ecological (dis)similarity in *Pennisetum* section *Brevivalvula*

Of each species typical samples can be distinguished morphologically and partly ecologically, but many samples cannot be classified with confidence because they have intermediate characters. *P. pedicellatum* is morphologically most differentiated. The species has more than one spikelet, of which at least one is pedicelled, per involucrum; the involucres have many bristles with long silky hairs; and there are relatively few involucres per panicle. The other species all have one sessile spikelet. Differences between these species are found mainly in the hairiness, the size and number of the involucrum bristles, the panicle length and the life cycle.

Characters such as plant height, number of inflorescences per plant or leaf length have not been used in this study, because the plants are day length sensitive. Inflorescence color is a striking feature in the section, and ranges from white to violet and deep purple, or in some species (reddish)-yellow. This character has not been used in the morphological analysis because precise color identification was not available at the time of collection, and the colors fade in herbarium material. Based on a coding system from the field, a preliminary principal component analysis was done including the inflorescence color but the character did not get a high factor loading, despite the predominantly yellowish color of *P. setosum* and *P. atrichum*. For the other species the variation in inflorescence color was very large.

Some niche differentiation has taken place in the section. *P. pedicellatum* is best adapted to the dry sahelian climate, but is also present in more humid climates, in lower numbers, where rare perennial-like samples of this species can be encountered as well. The perennials *P. setosum*, and the rare *P. atrichum*, are more adapted to the humid tropics, represented by vegetation zones III and IV. Seeds, the only means of perpetuation of annuals, are better adapted to survive the hot and dry period of the Sahel, than perennial root systems, at least of grasses. Perennials also have the tendency to flower later than annuals, but have a competitive advantage over annuals at the beginning of the rainy season. Field and village margins, which are annually burned, are suitable both for annuals and perennials. *P. polystachion* and *P. subangustum* have been found from the dry sahelian zone, although in low numbers, to the humid coast. They are typical examples of generalists of the section, although they have their optimum in different zones. *P. hordeoides* is basically a glabrous-bristled version of *P. subangustum*. It is not a common species in our samples, and has its highes distribution in zone II, the zone with most morphological and genetic variation.

Diploid-tetraploid-haploid cycles and *Pennisetum* section *Brevivalvula*

The *Bothriochloa-Dichanthium* agamic complex consists of several sexual diploid species plus a complex structure of largely apomictic polyploids. There are 14 diploid communities among 600 populations sampled in the complex, extending from Senegal to Singapore. The diploids are found only in part of the distribution area, and form a more or less continuous variable complex with the polyploids in that area. The polyploids, which are not in contact with the diploids, are essentially obligate apomicts, and are less variable than the mixed populations (De Wet, 1968).

In this complex partially sterile but fully sexual tetraploids, and largely sterile triploids, are produced at a low level by diploids that regularly form some diploid gametes that may fertilize, or may be fertilized by, diploid or haploid gametes. These tetraploids may provide a temporary genetic bridge between apomictic types, because they produce normal pollen. The numerical superior apomicts probably swamp these sexual tetraploids fast. The progeny of a facultative apomictic tetraploid includes mostly maternal tetraploids, some tetraploid and hexaploid hybrids, and some haploids. Most of the haploids are sterile, but the rare fertile ones could become established, thus playing some role in the evolution of apomictic grasses (De Wet & Harlan, 1970).

The diploid populations probably often get outnumbered by the apomicts, but seem to survive long enough to produce some sexually fertile tetraploids, which in their turn produce sufficient haploids to maintain a small diploid sexually fertile colony. This would also explain the widely scattered, disjunct distribution of diploid colonies, and the limited numbers within these colonies of the largely tetraploid agamospecies. Some of the scattered diploid populations in the complex could be regarded as ancestral relicts of the tetraploid ecotype as well.

Bothriochloa intermedia is a special case in this complex because it seems to be largely existing of introgression products. The compilospecies concept has been introduced by Harlan & De Wet (1963), to typify agamospecies that exist mainly of genotypes of related species. They can even cause the extinction of those related species, because they have become assimilated completely. In this sense, compilospecies are genetically aggressive, a characteristic often associated with a tolerance for disturbed habitats.

The diploid-tetraploid-haploid cycles as described above could well be a mechanism to release genetic variation in *Pennisetum* section *Brevivalvula* as well. Although only one diploid population has been found until now, other such populations probably do exist in West Africa. It is difficult to know whether these diploids are formed as haploids from sexual reproducing tetraploids, or whether they are ancestors of the polyploid complex. The size of the diploid population gives the impression that it is not being swamped by polyploid apomicts, although introgression occurs.

P. polystachion seems to be the most variable species in the complex. It has a large morphological overlap with the other species of the section, and mostly with *P. subangustum* and *P. setosum*. It has the largest geographical distribution, and all ploidy levels known in the section are present here, including sexual diploids. Based on these characteristics, *P. polystachion* makes a good candidate as a compilospecies, restricting species formation in the section.

Species concepts

The species description used throughout this thesis is a purely practical one, based on morphological distinguishing characters. It is close to the morphological species concept, also called the classical or the Linnean species concept, where the species are the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means (Cronquist, 1978). In *Pennisetum* section *Brevivalvula* the distinction between the different species is vague, because of hybridization events or recent genetic isolation.

Because *Pennisetum* section *Brevivalvula* is characterized by apomixis, the agamospecies concept would seem the correct approach to use. The identification of all different clones as microspecies would be unsatisfactory though, because in this complex many plants are facultative apomicts, and diploids exist as well, so new genotypes are produced in nature all the time. In complexes with higher levels of obligate apomixis, like in *Rubus* and *Taraxacum*, so many microspecies have been recognized that it has become an unworkable situation.

A compromise can be made, where microspecies are grouped into morphological entities, and be recognized at the species level, or, as Brunken has done, at subspecies level. Personally I would prefer to keep the morphological taxa at species level because the hybridization processes probably take place among all species, and not only between subspecies of species.

Possibilities for further research

The present study gives openings for a variety of possible studies related to different aspects of this agamic species complex.

Firstly, there are questions left unanswered related to the genetics of the section. How many apomixis genes are involved, and how are they regulated in the complex? How is the sexual-apomixis balance regulated in each plant? What is the role of the diploids in releasing genetic variation in the complex? Answers to these questions could be found through inheritance studies by crossing experiments between diploids and polyploids. These studies are difficult because apomixis has been shown to be controlled by different mechanisms both within and between species and genera.

Secondly, there are questions concerning the population dynamics of the section. How can diploids and polyploids exist in sympatry, or polyploids from different species? Which solutions did they find for niche differentiation? Do hybridization events take place preferentially between certain species? Are certain clones more successful than others are, and if so what is the reason for this to happen? What is the phenetic plasticity of a genotype? Answers to these questions can be found in competition tests comparing different species, or cytotypes of the same species (different polyploids or polyploids versus diploids). Differences in ecological preferences, and phenotypic expression can be found by testing different soil, water, nutrient and light regimes.

Thirdly, there are agronomic interests. Which characteristics of *Pennisetum* section *Brevivalvula* are of interest of agronomic improvements? How can apomixis play a role in the development of better performing crops?

Comments in the use of *Pennisetum* section *Brevivalvula*

Pearl millet farmers in Niger and in other sahelian countries are subsistence farmers, living sandwiched between the dry pastoral sub-Saharan zone in the North, where rainfall patterns are too irregular and sparse to cultivate crops, and between the more humid, and (almost) overpopulated South. Towards the South, pearl millet is gradually replaced by the higher producing sorghum, which is more sensitive for dry spells and quality of the soil. Large areas of the sahelian zone are characterized by poor sandy soils, and pearl millet is the only crop well adapted to these conditions. The cultivation of pearl millet is very extensive, in order to allow the plants to develop under optimal water and nutrient conditions.

Pearl millet is part of a crop-weed complex, where the wild, cultivated and weedy hybrid forms partly coexist. In the northern pastoral zone descendants of the wild ancestral form grow allopatric from the cultivated form, but in farmer's fields more to the south, hybrids are abundant, and pure wild forms are scarce. Farmers recognize the hybrids readily, but they deal with them differently, depending on the situation in which they live. In the south, where chances on normal, annual, harvests are high, the hybrids are weeded out, first at plantlet stage, and later the escaped ones before flowering, to avoid cross pollination with the cultivars. The real subsistence farmers leave the hybrids in the fields, because they are harvested and eaten before the cultivated millet is harvested, if it is harvested at all. Those farmers live at the fringe of the pastoral zone but this fringe is nowadays extended more to the south, because the annual rainfall has diminished and has become more irregular the last 20 years, causing regular harvest failures.

Incorporating specific genes in cultivated millet, such as the apomixis gene(s), seems a useful aim, in order to assure purebred lines, without pollution through cross-pollination. This apomictic pearl millet would seem useful only, if the new cultivars

would be a mixture of different clones, to prevent the wiping out of a crop by a disease or pest. For marginal areas, apomictic pearl millet cultivars would only be a solution if they are very drought tolerant and have a very short life cycle.

Attempts to introduce apomixis from wild relatives into pearl millet have until now resulted only in partially fertile apomictic plants with added chromosomes, which are unsuitable for agronomic purposes (Ozias-Akins et al., 1993). Because of conflicting and incomplete evidence on the genetic base of apomixis, there is no consensus about the feasibility of two breeding approaches, transfer versus synthesis. Interspecific hybridization was used as a tool for the introduction of apomixis into pearl millet from *P. squamulatum*. This species is hexaploid, but could be hybridized with tetraploid breeding lines of *P. glaucum* (Dujardin & Hanna, 1983, 1989). Among backcross progenies onto pearl millet apomictic types were obtained. It seems therefore likely that gene(s) for apomixis are localized on a small part of the genome of the donor species.

Other restrictions in the feasibility of producing apomictic pearl millet cultivars are (1) pearl millet is a diploid; advantages of apomixis are related to polyploidy, (2) apomixis has proved to be successful mostly if they are facultative apomicts, which would not be an option for pearl millet, (3) pearl millet should be able to produce without much extra nutrient input, because fertilizers are too expensive for subsistence farmers, and manure deposition is very localized in these extensive farming systems. Perhaps better perspectives can be found for breeders in the incorporation of resistance genes for diseases or for plant parasites, like *Striga hermonthica* (Del.) Benth. This *Striga* is extending its distribution area at a fast rate, because the soils are becoming poorer, thus the millet plants are weaker, and easier to attack. Breeders have not found any satisfactory results in their search for *Striga* resistance in pearl millet, until now. In some preliminary studies (see Chapter 1) with species from section *Brevivalvula*, *Striga* does not develop on plants from certain origins. Possibly there is some reason for optimism here, despite the fact that pearl millet and the *Brevivalvula* section are from completely different gene pools.

Another perspective is the breeding for green fodder improvement. Livestock and especially ruminants are an essential component of most of the agricultural production systems in sub-Saharan Africa. In the drier areas, the quantity of natural forages is often insufficient, whereas in the wetter areas the feed supplies are usually ample but their protein and energy concentrations are low and they are therefore of poor quality. *P. pedicellatum* would be a good candidate in the Sahel as feed for cattle.

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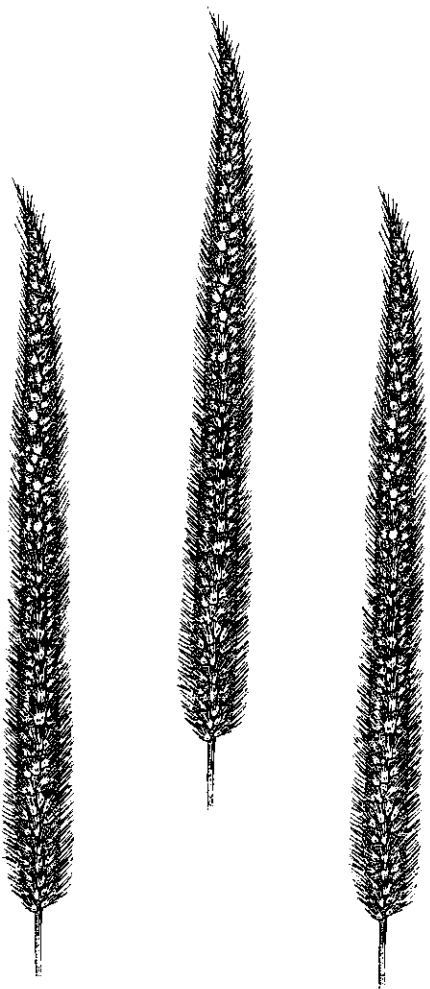

Pennisetum subangustum

Summary

The genus *Pennisetum*, bristle grass, is widely distributed throughout the tropics. The best known species is *P. glaucum*, pearl millet, which is the most drought tolerant cereal of semi-arid Africa and India. Wild relatives of pearl millet are of interest especially as sources for apomictic gene(s), sources for disease resistance, and as fodder improvement. *Pennisetum* section *Brevivalvula*, a group of wild species that are sympatric with pearl millet, reproduces predominantly by apomixis. A better understanding of the distribution patterns of the species in relation to the functioning of apomixis in this complex, would be of help for the current research in this field.

This thesis deals primarily with the morphological and isozymic patterns found in *Pennisetum* section *Brevivalvula* in West Africa, in relation to the reproduction systems present and the geographical distribution. The results of the different chapters will be summarized chronologically in this chapter.

The first chapter is a literature review of the five sections present in *Pennisetum*. Section *Brevivalvula* is the most coherent section in the genus, but distinction at species level is difficult. Six morphological species have been recognized for a long time: *P. atrichum*, *P. hordeoides*, *P. pedicellatum*, *P. polystachion*, *P. setosum* and *P. subangustum*. *P. atrichum* and *P. setosum* are perennials, the others are annuals. This number has been reduced to three species and a number of subspecies, after a revision, because of overlapping morphological characters.

Section *Brevivalvula* originated in tropical Africa, and more specifically in West Africa, because all species are encountered here. Most species have been introduced from Africa, on purpose or not, to other parts of the world. *P. atrichum* is distributed throughout tropical Africa only, *P. hordeoides* and *P. subangustum* have a smaller distribution area in Africa than *P. atrichum*, but are also present in a small area of India. *P. pedicellatum*, *P. polystachion*, and *P. setosum* are distributed throughout most of the tropics of the old world, while *P. setosum* is also introduced into large parts of the new world. *Brevivalvula* species occur mainly in anthropogenic areas, such as field boundaries, roadsides, and margins of villages.

Four basic chromosome numbers are present in *Pennisetum*, x = 5, 7, 8, and 9. Pearl millet, the only species in the primary gene pool, is a diploid (x = 7) and elephant grass, the only species in the secondary gene pool, is a tetraploid (x = 7). The other *Pennisetum* species, section *Brevivalvula* included, are in the tertiary gene pool of the cultivated species, and mostly have x = 9. Four ploidy levels are found in the section. Tetraploids were found in all species, hexaploids in most species, except in *P. atrichum*. Pentaploids were found only in *P. pedicellatum* and *P. polystachion*, and diploids were only found in *P. polystachion* and *P. subangustum*. Later, see Chapter 4, pentaploids were found also in *P. setosum* and *P. hordeoides*, from samples from Ivory Coast. This variation in ploidy levels is connected with the reproduction system

present in most of the section. According to the literature, agamospermy, in the form of 4-nucleate apospory with pseudogamy, has been found in the polyploid cytotypes of all species. Diploid taxa of agamic complexes are considered to reproduce sexually; this has been proved in Chapter 2 and 3.

In Chapter 2 the sampling strategy for the section over part of its center of diversity has been described. For 304 plants of the section, originating from Burkina Faso, Benin and southern Niger, ploidy levels were assessed by DAPI-flow cytometry. The results were confirmed for 54 plants by chromosome counts. The geographical distribution of the ploidy levels appears related to major vegetation zones, where zone I is the dry sahelian zone and zone III the coast of Benin. In zone I predominantly tetraploids were found, in zone III predominantly hexaploids, and in zone II all four ploidy levels were encountered. The species were also distributed unevenly over the zones: *P. pedicellatum* was observed only in the north of the study area, zone I and II; *P. hordeoides* was predominantly observed in zone II; *P. setosum* was observed in zone II and III, while *P. polystachion* and *P. subangustum* were observed in the whole sampled area. Diploid populations of *P. polystachion* and *P. subangustum* were only found in the Banfora area, in Burkina Faso. A preliminary study of embryo sacs showed sexual reproduction for a diploid *P. subangustum* sample, and showed aposporic embryo formation, in tetraploid samples of *P. polystachion*, *P. hordeoides* and *P. pedicellatum*.

Chapter 3 shows the results of the genotypic variation found in the progeny of 118 genitors belonging to five species of *Pennisetum* section *Brevivalvula*. The variation has been estimated by isozyme electrophoresis with observations of five enzymatic systems, in order to compare the type of reproduction in polyploid and diploid chromosomal taxa. A total of 112 different 5-locus genotypes has been found, over all taxa. All progeny of the diploid populations of *P. polystachion* and *P. subangustum* showed genotypic variation, as a consequence of their sexual reproduction system. At the polyploid level the type of reproduction appears to be predominantly apomictic, although genotypic variation was found in the progeny of 9% of the genitors. Genetic relationships have been observed between the diploid sexual *P. polystachion* and *P. subangustum*, and, to a lesser extent, with the tetraploids of the same taxa as well. Tetraploid *P. polystachion* and *P. pedicellatum* share genotypes with most other chromosomal taxa.

The biogeography and morphological variation found in *Pennisetum* section *Brevivalvula* samples from Benin, Burkina Faso, southern Niger and Ivory Coast, are analyzed in Chapter 4. A fourth vegetation zone, the humid south of Ivory Coast, has been added to the three zones used in Chapter 2, as a basis for geographical differentiation between the species. Because the sampling method is biased, the results can only be the interpreted for the samples, not for the species in general, although tendencies can be seen. The results from the ploidy levels analysis from the samples of

Ivory Coast were used together with the ploidy levels already obtained from the other samples (Chapter 2). The tetraploid ploidy level was by far the most common level, followed by the hexaploid ploidy level, while the diploid and the pentaploid ploidy levels were scarce. Tetra- and hexaploids were found in all zones, pentaploids only in zone I and II, and diploids only in zone II. In zone I significantly less hexaploids were found than in the other zones, while in zone III significantly more hexaploids were found than in zone II. The other zones did not show any significant differences.

Four new chromosomal taxa were found in the samples from Ivory Coast, adding up to a total of 17 chromosomal taxa in all of the samples. *P. polystachion* and *P. subangustum* are the most common species in the samples, followed in decreasing order by *P. pedicellatum*, *P. setosum*, *P. hordeoides* and finally *P. atrichum*.

Some patterns are found for the geographical distribution of the samples over the vegetation zones. Most species are found in all zones, but the quantitative distribution of the species over these zones is different. *P. pedicellatum* occurs in significantly higher numbers in zone I than all the other species. The remaining species are combined in their predominance over the other zones: *P. polystachion* and *P. hordeoides* occur in significantly higher numbers in zone II; *P. hordeoides* and the perennial *P. setosum* in zone III; *P. subangustum* and *P. setosum* occur in significantly higher numbers in zone IV. The other perennial species, *P. atrichum*, was found in zone III only, while *P. setosum* was missing completely from zone I.

Significant differences exist between part of the cytotypes of the species. *P. setosum* has significantly more hexaploids than the other species. *P. hordeoides* and *P. subangustum* consist mainly of tetraploids, and differ significantly from *P. pedicellatum* and *P. polystachion* for having less hexaploids. *P. subangustum* has significantly fewer diploids than *P. polystachion*.

Nineteen characters were used in a principal component analysis (PCA) using 614 individual samples from the species of section *Brevivalvula*. A second PCA was done without the samples of *P. pedicellatum*, using 16 characters, to allow a better scattering of the remaining samples. Distinctive characters were life cycle, number of spikelets, presence of pedicel, and length of bristle pubescence, the other characters are overlapping (widely), although significant differences exist in general between the means of those characters. The group of *P. pedicellatum* samples was almost separated from the other species in the first PCA, because of differences in the following characters: (normally) more than one spikelet, presence of a pedicel, more and longer involucrum bristles with longer bristle pubescence, and fewer involucres per inflorescence.

The second PCA, of the group without *P. pedicellatum*, shows extensive overlap between the morphological species, but some patterns are present. There is a continuous scale with increasing values for the following characters from left to right: life cycle, panicle length, terminal bristle length and ratio between terminal bristle length and height of bristle pubescence. *P. hordeoides* is located completely on the left-hand side of this scale, *P. setosum* completely on the right, and the center is

formed by *P. subangustum* (more to the left) and *P. polystachion* (more to the right). When the ploidy levels instead of the species are plotted in this PCA, the hexaploids are found to be situated mainly on the right half of the scale, and is the predominant ploidy level completely at the right side of the scale. This hexaploid side coincides with the species *P. setosum*. The diploids are found at the right side of the scale, close to the transition zone between the tetraploids and the group of the pure hexaploids, indicating that they are intermediate between the tetraploids and the hexaploids for the characters with high factor loading.

In Chapter 5 the genotypic variation present in 635 samples of *Pennisetum* section *Brevivalvula* has been analyzed by isozyme electrophoresis, resulting in 146 different 5-locus genotypes from combinations of 26 alleles. Of these genotypes 57% occurred as unique patterns in only 13% of the genitors. The remaining 43% of the genotypes was shared between different species, chromosomal taxa or sites. Tetraploid *P. pedicellatum*, *P. polystachion*, *P. subangustum* and *P. hordeoides*, and hexaploid *P. setosum* are the most common chromosomal taxa.

The mean number of alleles (A) and the mean observed heterozygosity (Ho) are significantly lower in the diploids than in the polyploids, but the expected heterozygosity (He) for the diploids was not significantly different from Ho, indicating that they are outcrossing. A and Ho were not significantly different between the morphological species, nor was there a geographical effect.

Multivariate correspondence analysis has been used as a method to evaluate which of the different frequencies, based on the presence or absence of the 20 most common alleles, are correlated with the variation in the following categories: morphological species, ploidy levels and geographical zones. All species were found to be distinct from each other by having significantly more or less of some alleles. *P. polystachion* was also different because of the presence of 6 rare alleles. Significant differences were found between all ploidy levels, and there was also a geographical effect.

The results of the analysis of the 5-locus genotypes of the samples of *Pennisetum* section *Brevivalvula* do reasonably agree with the results of the morphological analysis from Chapter 4. The species show extensive overlap for most morphological characters, but some structure exists, allowing a certain differentiation at species level. This pattern is strengthened by differences in ploidy level and geography (ecology). The results of the isozyme study confirm these patterns, based on different proportions of 20 alleles. The samples observed all seem to share the same gene pool.

In Chapter 6 the main results found in this study of *Pennisetum* section *Brevivalvula* are discussed. The section is characterized by polyploidy and apomixis. Most agamic complexes known from the literature are perennial, increasing the chance of survival of sterile genotypes. Section *Brevivalvula* consists mainly of annual species. This annual life cycle excludes the possibility for perpetuating sterile genotypes, although the perennials in the section do not really fare better because they do not proliferate by vegetative means.

The samples of the species analyzed show several patterns. The first pattern is shown by the morphological analysis, mainly based on characters of the inflorescence. One species, *P. pedicellatum*, is clearly distinct from the other species. The remaining species are part of a large group, in which the taxa are distinguished on a horizontal scale of overlapping characters, from left to right: *P. hordeoides*, *P. polystachion*, *P. polystachion* and *P. setosum*. Of *P. atrichum* too few samples were collected to distinguish a pattern.

The second pattern in the group is formed by the different cytotypes. One of the species, *P. setosum*, is mainly hexaploid, the others are mainly tetraploid. Diploids are found in two species, *P. polystachion* and *P. subangustum*.

The third pattern is one of geographical (and ecological) differentiation. *P. pedicellatum* is the predominant species in the dry sahelian zone. The other species occur in different combinations of predominance over the other zones. In the second vegetation zone, which is the transition zone between the dry Sahel and the humid rain forest zone, all ploidy levels and most species are present, but *P. polystachion* and *P. hordeoides* are predominant here. More to the south, a predominance of *P. hordeoides* and *P. setosum* is alternated with a predominance of *P. setosum* and *P. subangustum*.

The fourth pattern in the group is formed by the 5-locus genotypes. More than 85% of the samples of different species are connected by patterns of identical genotypes. When the proportions of 20 alleles are compared between species, ploidy levels or geographical areas, significant differences are found everywhere. The results also indicate that the samples share the same gene pool.

Field botanists will have difficulties at times in determining the taxa in *Pennisetum* section *Brevivalvula* here circumscribed, because intermediates occur at a large scale in West Africa. Since the complex is basically reproducing by apomixis, large patches or populations composed of only a few clones can be encountered at a regular base as well. The active state of evolution of the complex renders a fully unambiguous description of the species of section *Brevivalvula* impossible.

Samenvatting

Kader van het onderzoek

Het plantengeslacht *Pennisetum*, borstelgras, is wijd verspreid in de tropen. De meest bekende soort is parelgierst, *P. glaucum*, bekend in Nederland vooral als vogelvoer. In de tropen, met name in de droge Sahel regio in Afrika en in India, is parelgierst van groot belang voor de lokale voedselvoorziening, omdat het het enige graangewas is dat onder deze droge omstandigheden kan groeien. Wilde verwanten van parelgierst zijn interessant voor onderzoek door de mogelijke aanwezigheid van resistentiegenen tegen diverse ziekten, door de aanwezigheid van genen die apomixie veroorzaken, en ook als veredeld voedergewas voor het vee.

Apomixie kan worden omschreven als de vegetatieve vermeerdering door middel van zaad. De zaden hebben dezelfde genetische samenstelling als de moederplant, hetgeen voordelen oplevert zoals een stabiel nakomelingschap zonder verandering of degeneratie door hybridisatie, en het fixeren van heterosis.

Een groep nauw verwante wilde soorten van de parelgierst, de sectie *Brevivalvula*, is in dit proefschrift bestudeerd. De soorten in deze sectie hebben ten dele een zelfde verspreiding als parelgierst. Het onderzoek richtte zich vooral op de morfologische en genetische variatie van de soorten in West Afrika, in samenhang met de voortplantingsmechanismen en de geografische verspreiding.

De soorten in *Pennisetum* sectie *Brevivalvula*

Zes morfologische soorten worden sinds langere tijd onderscheiden in de sectie: *P. atrichum*, *P. hordeoides*, *P. pedicellatum*, *P. polystachion*, *P. setosum* en *P. subangustum*. In recente artikelen is dit aantal gereduceerd tot drie soorten en een aantal ondersoorten, dat verschilt van het oorspronkelijke aantal soorten. Tijdens dit onderzoek zijn de taxa op soortsniveau gehandhaafd. *P. setosum* en *P. atrichum* zijn meerjarig, de overige soorten zijn eenjarig.

De soorten bestaan voornamelijk uit polyploiden die zich door middel van apomixie vermenigvuldigen. Tetraploiden zijn het meest algemeen, gevolgd door hexaploiden; pentaploiden zijn relatief zeldzaam. In *P. setosum* werden significant meer hexaploiden aangetroffen dan in de andere soorten. Diploiden, die zich sexueel vermenigvuldigen, zijn voor het eerst gevonden in de sectie tijdens een verzameltocht in het westen van Burkina Faso en werden alleen aangetroffen in *P. polystachion* en *P. subangustum*.

De meeste apomictische complexen zijn meerjarig, zodat steriele planten, die meestal een afwijkend aantal chromosomen hebben, kunnen blijven voortbestaan. De

sectie *Brevivalvula* bestaat echter voor een groot gedeelte uit eenjarige soorten. Daarnaast zijn veel apomictische complexen facultatief apomictisch, d.w.z. een klein gedeelte van de zaden wordt sexueel gevormd. In hoofdstuk 3 van dit proefschrift wordt aangetoond dat minstens 9% van de polyploiden van de sectie *Brevivalvula*, verdeeld over alle soorten, een klein aantal genetisch variabele nakomelingen heeft, mogelijk door residuaire sexuele vermenigvuldiging. De meeste nakomelingen van alle diploiden daarentegen vertonen afwijkende genotypes, een teken dat ze zich sexueel voortplanten.

Morfologische variatie in sectie *Brevivalvula*

Een morfologische analyse werd uitgevoerd met behulp van een principale componenten analyse, op voornamelijk stabiele kenmerken van de bloeiwijze. *P. pedicellatum* wijkt het meest af van de andere soorten doordat deze soort minder en grotere omwindsels heeft, met meestal meer dan één gesteeld aartje per omwindsel. De andere soorten lijken sterk op elkaar omdat de vele omwindsels dicht opeen gepakt zijn op de rachis, en elk omwindsel slechts één zittend aartje bezit. De analyse toonde verder dat de vier overige soorten een overlappende reeks vormen, uitgezonderd *P. atrichum*, omdat daar te weinig planten van zijn verzameld. Van links naar rechts in de reeks zijn dit de soorten *P. hordeoides*, *P. subangustum*, *P. polystachion* en *P. setosum*. *P. hordeoides* heeft weinig involucrumborstels, die kaal en meestal kort zijn zodat de aartjes zichtbaar zijn; *P. subangustum* heeft weinig involucrumborstels, die kort zijn maar ook kort behaard; *P. polystachion* heeft meer en langere involucrumborstels, die langer behaard zijn, en *P. setosum* heeft de meeste en langste involucrumborstels. Andere, meest vegetatieve verschillen tussen de soorten bestaan ook, maar zijn niet gebruikt in de analyse om logistieke redenen.

Verspreidingspatronen in sectie *Brevivalvula*

Omdat de planten per vindplaats gericht verzameld zijn, zodat een zo groot mogelijke morfologische variatie verkregen werd, kunnen de resultaten alleen met het nodige voorbehoud worden geinterpreteerd. Grote lijnen zijn wel zichtbaar.

De soorten zijn verzameld over vier grote vegetatiezones in West Afrika: zone I is de droge Sahelzone, zone II is de savannezone tussen het droge noorden en het vochtige regenbosklimaat, zone III is het drogere deel van het regenbosklimaat dat ook aan de kust van Benin voorkomt, terwijl zone IV het vochtige regenbos is dat tot aan de kust van Ivoorkust voorkomt.

De meeste soorten worden aangetroffen in alle zones, maar de kwantitatieve verpreiding verschilt sterk. *P. pedicellatum* komt significant vaker voor dan de andere soorten in de droge Sahelzone. De overige soorten combineren hun predominantie over

zones als volgt: *P. polystachion* en *P. hordeoides* komen meer voor in zone II, *P. hordeoides* en de meerjarige *P. setosum* komen meer voor in zone III, terwijl *P. setosum* en *P. subangustum* meer voorkomen in zone IV. *P. atrichum*, de andere meerjarige soort, werd alleen in zone III aangetroffen. *P. setosum* werd niet aangetroffen in zone I. Tetraploiden en hexaploiden worden in alle zones aangetroffen, pentaploiden alleen in zone I en II, en de diploiden alleen in zone II. Het aantal hexaploiden neemt toe van de Sahel richting de kust.

Genotypische variatie in sectie *Brevivalvula*

In 635 verschillende planten zijn 146 verschillende 5-locus genotypes gevonden uit combinaties van 26 allelen. Van deze genotypes kwam 57% slechts een keer voor, in 13% van de planten. De overige 43% van de genotypes kwam meerdere malen voor in verschillende soorten, cytotypes of vindplaatsen.

Het gemiddelde aantal allelen en de gemiddelde geobserveerde heterozygositeit zijn significant lager in de diploiden dan in de polyploiden. Er zijn geen verschillen gevonden voor deze gemiddelden tussen de soorten noch zijn er geografische verschillen. Proportionele verschillen in allelen werden wel gevonden tussen de soorten, ploidie niveaus en vegetatiezones.

Slotconclusie

Botanici zullen soms problemen ondervinden wanneer ze de soorten van *Pennisetum* sectie *Brevivalvula* in het veld willen herkennen, omdat intermediare exemplaren regelmatig voorkomen, in elk geval in West Afrika. Omdat het complex zich voor het grootste gedeelte voortplant door middel van apomixis, ontstaan er ook kleinere en grotere populaties van slechts een paar klonen. Daar waar sexuele diploiden voorkomen, worden in hoog tempo verschillende genotypes en fenotypes gevormd. Deze actieve staat van evolutie van het complex maakt een ondubbelzinnige beschrijving van de soorten in sectie *Brevivalvula* onmogelijk.

De handhaving van de taxa op soortsniveau is echter te verkiezen boven de indeling in soorten en ondersoorten, omdat zo niet de indruk gegeven wordt dat sommige taxa nauwer aan elkaar verwant zijn dan andere, en op deze wijze ook één- en meerjarige taxa niet als ondersoort van eenzelfde soort voorkomen.

Résumé

Le genre *Pennisetum* a une large répartition dans la zone tropicale. L'espèce la plus connue c'est le *P. glaucum*, le mil pénicillaire, qui est la céréale la plus tolérante à la sécheresse de l'Afrique et de l'Inde semi-aride. Les espèces apparentées au mil sont intéressantes comme sources des gènes apomictiques, comme sources de résistance contre certaines maladies, et pour la création des plantes fourragères. Les espèces sauvages de la section *Brevivalvula* du genre *Pennisetum* sont en sympatrie avec le mil pénicillaire, et reproduisent principalement par apomixie. Une meilleure compréhension des aires de repartition des espèces qui sont en relation avec le fonctionnement de l'apomixie dans ce complexe, aidera à la recherche actuelle de ce type.

Dans cette thèse, les groupes morphologiques et enzymatiques de *Pennisetum* section *Brevivalvula* en Afrique de l'Ouest sont étudiés, en relation avec les systèmes de reproduction et la distribution géographique.

La section *Brevivalvula* est la section la plus cohérente du genre, mais une distinction au niveau des espèces est beaucoup moins évidente. Six espèces morphologiques ont été reconnues avant une révision de la section: *P. atrichum*, *P. hordeoides*, *P. pedicellatum*, *P. polystachion*, *P. setosum* et *P. subangustum*. *P. setosum* et *P. atrichum* sont pérennes, alors que les autres espèces sont annuelles. Ce nombre d'espèces a été réduit ensuite à trois espèces et à quelques sous-espèces, à cause du chevauchement des caractères morphologiques: *P. hordeoides* reste une espèce séparée; *P. polystachion* est divisée en deux ou trois sous-espèces: *P. polystachion* ssp. *polystachion*, *P. polystachion* ssp. *atrichum*, et souvent *P. polystachion* ssp. *setosum*; *P. pedicellatum* est partagé en deux sous-espèces, dont une est nouvellement reconnue: *P. pedicellatum* ssp. *pedicellatum* et *P. pedicellatum* ssp. *unispiculum*. *P. subangustum* est devenu synonyme de *P. polystachion* ssp. *polystachion*. Dans la thèse, les taxa de *Pennisetum* section *Brevivalvula* sont distingués selon l'ancienne classification, afin de pouvoir reconnaître le plus de polymorphisme que possible.

La section *Brevivalvula* est originaire d'Afrique tropicale, et plus spécifiquement d'Afrique de l'Ouest, parce que toutes les espèces y sont en sympatrie. La plupart des espèces a été exportée vers d'autres parties du monde, d'une façon délibérée ou pas. *P. atrichum* a une large distribution en Afrique tropicale, *P. hordeoides* et *P. subangustum* ont une distribution plus restreinte en Afrique mais ils sont présents aussi dans une petite zone en Inde. *P. pedicellatum*, *P. polystachion* et *P. setosum* sont distribuées sous tous les tropiques de l'ancien monde, alors que *P. setosum* a été introduit aussi au nouveau monde. On peut rencontrer les espèces de la section

Brevivalvula plutôt dans des zones anthropisées, comme les bords de champs et les villages, et les bords de routes.

Quatre niveaux de ploidie (x = 9) ont été trouvés dans la section. Les tétraploides sont les plus communs, suivis par les hexaploides. Peu de pentaploides et diploides ont été rencontrés. Cette variation du niveau de ploidie est en relation avec le système de reproduction présent dans la plupart des espèces de la section. L'agamosporie, dans la forme d'aposporie à 4 noyeaux, avec pseudogamie, a été trouvée dans les cytotypes polyploides de toutes les espèces. Les diploides étaient considérés d'avoir un système de reproduction sexué; ceci a été prouvé dans les Chapitres 2 et 3. Tous les diploides et 9 % des polyploides ont montré la variation enzymatiques dans leurs descendances. Pour les polyploides cela indique une sexualité résiduelle, ou bien, d'une apomixie facultative.

La biogéographie et la variation morphologique des échantillons en provenance du Bénin, du Sud-Niger, du Burkina Faso et de la Côte d'Ivoire ont été analysées dans le Chapitre 4. La distribution géographique des espèces et des niveaux de ploidie semble d'être en relation avec 4 grandes zones de végétation: la zone I est la zone sèche sahélienne, la zone II est la zone de savane entre le Nord sec et le Sud humide, la zone III est la côte humide du Bénin et la zone IV la côte plus humide de la Côte d'Ivoire. La plupart des espèces a été trouvée dans toutes les zones, mais leur distribution quantitative est différente: *P. pedicellatum* est l'espèce prédominante dans la zone I, les autres espèces sont combinées dans leur prédominance dans les autres zones. *P. polystachion* et *P. hordeoides* se trouvent en plus grands nombres dans les échantillons de la zone II, *P. hordeoides* et l'espèce pérenne *P. setosum* dans la zone III, et enfin *P. setosum* et *P. subangustum* dans la zone IV.

Pour les niveaux de ploidie, les tétraploides et les hexaploides sont observés dans toutes les zones, les pentaploides seulement dans les zones I et II, et les diploides seulement dans zone II. Dans la zone I on a trouvé significativement moins d'hexaploides, et dans la zone III on trouve significativement plus d'hexaploides qu'en zone II.

Pour la combinaison espèces/niveaux de ploidie, *P. setosum* a plus d'hexaploides que les autres espèces, *P. hordeoides* et *P. subangustum* présentent plutôt des tetraploides. *P. subangustum* et *P. polystachion* sont les seules espèces ou les diploides sont trouvés.

L'analyse en composantes principales montre que *P. pedicellatum* est l'espèce la plus divergente du groupe, ce qui correspond avec sa différence écologique. Les autres espèces forment un continuum d'espèces, basé sur les caractères suivants: longueur du cycle, longueur de la panicule, longueur du poil de l'involucre le plus long et hauteur de l'implantation des soies des poils. *P. hordeoides* est à une coté du continuum, et *P. setosum* à l'opposé. Au milieu, on trouve *P. subangustum*, vers *P. hordeoides*, et *P. polystachion*, vers *P. setosum*.

Dans le Chapitre 5, la variation génotypique observée dans les échantillons de la section est analysée par electrophorèse enzymatique: 146 génotypes différents pour 5-locus et 26 allèles. De ces génotypes, 57% apparaîssent comme des patterns uniques, dans 13 % des géniteurs. Les 43% qui restent, sont partagés parmi les différentes espèces, cytotypes ou sites de collection.

Le nombre moyen d'allèles (A) et l'hétérozygotie moyenne observé (Ho) sont plus élevés aux polyploides qu'aux diploides. Ho et A ne sont pas différents entre les espèces ou entre les zones géographiques. Les 26 allèles sont trouvés dans la zone II, les autres zones ont en moyenne 17 allèles.

Une analyse factorielle des correspondances a été utilisée comme méthode pour montrer les allèles qui sont corrélés avec la variation dans les catégories espèce, niveau de ploidie et zones géographique. Il apparait que chaque catégorie a des proportions différentes d'allèles. Ces résultats confirment globalement les résultats de l'analyse morphologique. Les caractères des espèces se chevauchent souvent pour une partie, mais pourtant il y a des patterns existent, qui admettent une certaine différentiation au niveau des espèces, ce qui est fortifié par les différences au niveau de ploidie et géographique. Les résultats de l'étude enzymatique confirment ces patterns, basés sur les proportions différentes des 20 allèles. Les échantillons observés semblent partager le même pool des gènes.

Le dernier chapitre traite les résultats obtenus dans la section *Brevivalvula* du genre *Pennisetum*. La section est caractérisée par la polyploidie et l'apomixie. La plupart des complexes agamiques connus dans la littérature sont des pérennes, ce qui augmente la chance de survie pour les génotypes stériles. La section *Brevivalvula* est constitué principalement d'espèces annuelles. Ce cycle de vie exclut la possibilité de propager des génotypes stériles, mais les pérennes du groupe ne font pas mieux, parce qu'ils ne se reproduisent pas végétativement.

Les échantillons analysés montrent des regroupements différents. Le premier regroupement est constitué à partir de l'analyse morphologique, basé en grande partie sur les caractères, de l'inflorescence. Une espèce, *P. pedicellatum*, est clairement différente d'autres espèces. Les autres espèces forment un grand groupe, dans lequel les taxa peuvent être reconnu sur une échelle de caractères qui se chevauchent, de gauche à droit: *P. hordeoides*, *P. subangustum*, *P. polystachion* et *P. setosum*. De *P. atrichum* trop peu d'échantillons ont été collectés pour distinguer un pattern.

Le deuxième regroupement est formé sur la base des cytotypes différents. Une espèce, *P. setosum*, est hexaploide en grande partie, les autres sont plutôt des tétraploides. Des diploides sexués sont trouvés dans deux espèces, *P. polystachion* et *P. subangustum*.

Le troisième regroupement est basé sur la différentiation géographique (et écologique). *P. pedicellatum* est l'espèce prédominante de la zone sèche sahélienne. Les autres espèces ont des combinaisons différentes sur les autres zones. Dans la

deuxième zone, qui est la zone de transition entre le Sahel sec et la côte humide des forets tropicales, tous les niveaux de ploidie et la plupart des espèces sont trouvés, mais *P. polystachion* et *P. hordeoides* sont prédominant dans les échantillons. Plus vers le Sud, une prédominance de *P. hordeoides* et *P. setosum* est alternée avec une prédominance de *P. hordeoides* et *P. subangustum*.

Le quatrième regroupement est formé sur la base des génotypes enzymatiques. Près de 85% des échantillons des différentes espèces ont des combinaisons alléliques identiques pour ces 5-locus. Quand on compare les proportions des 20 allèles (la comparaison entre les espèces, les niveaux de ploidie et les régions géographiques) on peut trouver des différences significatives partout. Les résultats montrent que les espèces appartiennent au même pool de gènes.

Les botanistes n'auront pas beaucoup de difficultés pour déterminer les espèces de *Pennisetum* section *Brevivalvula* décrites ici, parce que les formes intermédiaires sont présentes à l'échelle d'Afrique de l'Ouest. Comme le complexe se reproduit principalement par apomixie, les grandes surfaces couvertes par une seule espèce (probablement de clones) peuvent aussi être rencontrées. L'état d'évolution active du complexe, rend une description non ambigüe des espèces de la section *Brevivalvula* impossible.

Curriculum Vitae

Gabriëlla Harriët (Gaby) Schmelzer werd geboren op 20 februari 1964 te Vlissingen. Zij behaalde in 1982 haar v.w.o.-diploma aan de Comenius scholengemeenschap te Capelle aan de IJssel en begon vervolgens aan haar studie Biologie aan de Landbouwuniversiteit Wageningen. In 1986 werd een gecombineerd afstudeervak en stage doorgebracht in het regenbos van Ivoorkust en in 1988 een gecombineerd afstudeervak en stage bij het ICRISAT in Niger. In november 1988 behaalde zij haar doctoraalexamen met als afstudeervakken Plantentaxonomie, Onkruidkunde en Grondbewerking.

In 1989 voerde zij gedurende 5 maanden een vegetatiekartering uit bij ICRISAT in Niger. Vervolgens werkte zij vanaf maart 1990 anderhalf jaar als "horticulturist" voor de VSO (Voluntary Service Overseas) in de Malediven.

In november 1991 kreeg zij een positie bij DGIS als assistent-deskundige Onkruidkunde bij het DFPV (Département de Formation en Protection des Végétaux) in Niger, waar zij tot juni 1997 werkte. Begin 1993 kreeg zij daar de kans om, ten dele geïntegreerd met haar baan, promotieonderzoek te doen in samenwerking met het ORSTOM in Niger en de Landbouwuniversiteit te Wageningen, met als resultaat dit proefschrift.

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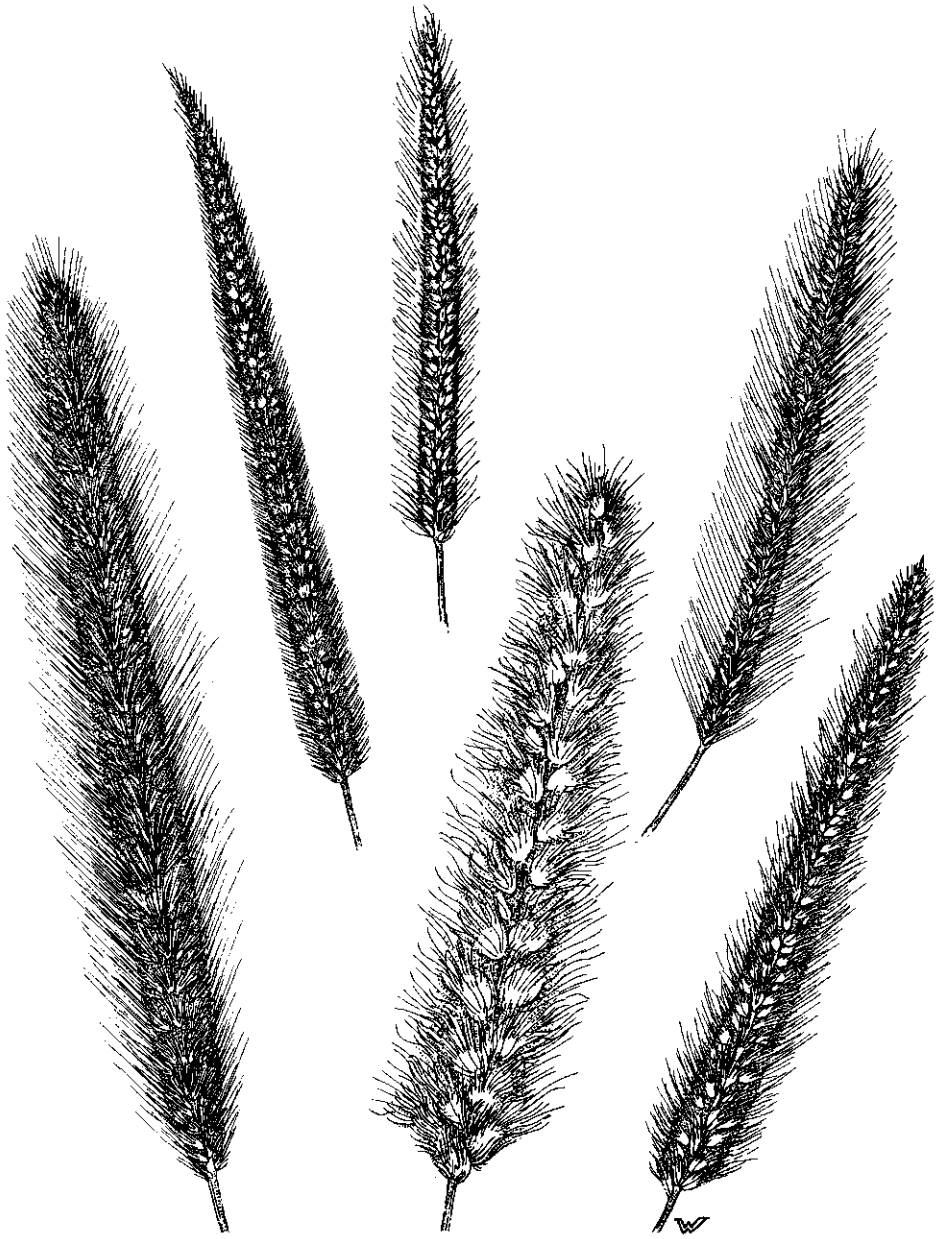

Line drawings of the inflorescences of the species studied. Starting at the bottom on the left side and then continued clockwise: *P. setosum*, *P. subangustum*, *P. hordeoides*, *P. atrichum*, *P. polystachion* and *P. pedicellatum*.