Genetic variability in the agamic species complex of *Pennisetum* section *Brevivalvula* (*Poaceae*) originating from West Africa : observations of ploidy levels and isozyme polymorphism

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Key Words : apomixis, *Brevivalvula*, flow cytometry, isozyme polymorphism, *Pennisetum*, polyploidy.

Abstract

Some characteristics of the species complex *Pennisetum* section *Brevivalvula* are polyploidy and apomixis. Four euploidy levels (x = 9) were assessed by DAPI-flow cytometry for 304 plants of the section, 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 the ploidy levels seems to be related to major ecological zones of West Africa, the zone with relief presenting a higher ploidy diversity than the others. Here, diploid populations of the annual species *P. polystachion* and *P. subangustum* were found. Genotypic variability expressed by the isozymic polymorphism does not show any significant differences between the diploid, sexual populations and the polyploid, apomictic populations of these two species.

Introduction

Polyploidy and apomixis are relatively common phenomena in flowering plants. Especially *Compositae*, *Rosaceae* and *Poaceae* are characterized by a rather high frequency of apomixis. Of the *Poaceae* the tribes *Paniceae* (including the genus *Pennisetum*) and *Andropogoneae* have the highest number of apomictic species (Brown & Emery 1958). The interest of using apomixis, or asexual seed production, in plant breeding has grown immensely over the last decade. The problems concerning improvement of agronomic characteristics such as yield, resistance to diseases, drought tolerance, as well as the stabilization of the heterosis effect in hybrid cultivars offer a great challenge for increasing and stabilizing the world food production. Wild relatives (ancestral and other close species) of crops dispose of one or sometimes more apomixis genes which can be transferred to cultivated varieties by crosses and backcrosses (Asker & Jerling 1992). The main problem is sterility, depending on the genetic distance between the species. In most crosses of this type, the sexual cultivar is diploid, and the apomictic wild species polyploid. Improvements in this type of research have been made lately in maize. In Mexico, at CIMMYT, a *Tripsacum* program started in 1989, in collaboration with ORSTOM, in order to isolate the apomixis gene. Hybrids between maize (2n = 20) and Tripsacum (2n = 72) have been made, followed by a series of back crossings, reducing the chromosome number to 2n = 28, in 1995. The identification of RFLP markers of the apomixis genes helped to select the plants with these genes at each backcrossing (Savidan, 1995). Another tropical species where reseach has been concentrated on for years is *Pennisetum glaucum* (L.) R. Br., pearl millet. It is an important crop, both for food and/or forage, in Subsaharan Africa, India and in the US. It is a sexual diploid (2n = 14). The potential of crossing wild, apomictic relatives with pearl millet has been investigated over the years. Successful crosses have been made between *P. glaucum* and *P. purpureum* (Burton 1944), *P. orientale* (Dujardin & Hanna 1983), *P. squamulatum* (Hanna & al. 1993), *P. setaceum* (Hanna

1979), *P. schweinfurthii* (Hanna & Dujardin 1986). No interspecific hybrids were produced, in the samples available, between pearl millet *P. glaucum* and *P. ramosum*, *P. mezianum*, *P. macrourum*, *P. pedicellatum*, and *P. polystachion*. However, there was partial seed development in diploid pearl millet x *P. pedicellatum* and *P. polystachion* (Dujardin & Hanna 1989).

The genus *Pennisetum* has been divided into 5 sections : *Gymnothrix*, *Eu-Pennisetum*, *Pennicillaria*, *Heterostachya*, and *Brevivalvula* and consists of some 120-130 species worldwide, in tropical and warm regions, according to Stapf & Hubbard (1934).These authors cite 91 species in Tropical Africa, while Lebrun & Stork (1995) restrict the number to 39. Section *Brevivalvula* includes 6 taxa classified as species according to morphological criteria : *P. pedicellatum* Trin., *P. hordeoides* (Lam.) Steud., *P. atrichum* Stapf & Hubbard, *P. polystachion* (L.) Schult., *P. subangustum* (Schum.) Stapf & Hubbard, and *P. setosum* (Swartz) L. Rich.

These species are supposed to be native of Tropical Africa. Not being able to exchange genes under natural conditions with the cultivated *P. glaucum*, they belong to the tertiary gene pool described by Harlan and de Wet (1971). However, their gene pool could contain characteristics like resistance to diseases, drought tolerance and apomixis which could serve to improve pearl millet. All species occur mainly in anthropic areas (Clayton 1972) as weeds, but are differentiated by their ecology, phenology, ploidy level and reproduction system (Gupta & Minocha 1980). *P. pedicellatum*, *P. polystachion* and *P. setosum* are widely used as green fodder for cattle, cut just before flowering (Skerman & Riveros 1990). *P. setosum* and *P. atrichum* are perennial and polyploid, while the other species are annual and polyploid. Brunken (1979) and Jauhar (1981) have made summaries of all chromosome numbers reported for *Pennisetum* section *Brevivalvula* (x = 9) : *P. setosum* (2n = 53, 54, 56, 78), *P. atrichum* (2n = 36), *P. pedicellatum* (2n = 24, 36, 45, 48, 52, 54), *P. polystachion* (2n = 32, 36, 45, 48, 52, 54, 63), *P. subangustum* (2n = 24, 32, 36, 54), and *P. hordeoides* (2n = 18). The first author observed variation of ploidy within the different species without a geographical or ecological differentiation.

The study presented here emphasizes the genetic variability in the agamic species complex *Brevivalvula* by the analysis of ploidy levels, described in detail in Renno & al (1995), and enzymatic patterns, in a sample collected from a part of the species distribution area, but through distinct ecological zones such as plains, areas with hills, and the coastal region.

Materiel and methods

Identification of taxa

The different taxa of sect. *Brevivalvula* were determined by using the botanical identification covering the largest polymorphism. Because the diagnostic characteristics are overlapping, the taxa are difficult to classify unequivocally. Six taxa here considered as "morphological species" have been retained for the study : *P. pedicellatum*, *P. hordeoides*, *P. polystachion*, *P. subangustum*, *P. setosum*, and *P. atrichum*.

Method of sampling

The geographical distribution of sect. *Brevivalvula* is too large to fully assess its polymorphism. In order to estimate the variation of ploidy levels, an "ecological strategy of collection" was chosen, which consisted of sampling through a maximum of biotopes. Two sampling transects were made by car, following existing roads. One transect ran from east to west, from southern Niger to west Burkina Fasa, and was mostly confined to the sahelian zone. The second transect was oriented north-south, starting in southern Niger, and crossing Benin from the sahelian zone to the relatively humid coastal zone through the Atakora hills.

Seventeen sites were chosen according to the maximal observed taxonomic diversity, and approaching the geographical distribution pattern of the species in the studied zone. To best represent the morphological diversity, about 20 individual plants were collected and identified for each site. The sampling method of the seeds allow quick access to morphological variability but tend to

overestimate the real polymorphism of populations, because certain possibly dominant genotypes are not representatively sampled. For each seed lot sampled for a genitor *in situ*, one progeny was analysed at plantlet stage by flow cytometry and for part of the sample another progeny was analysed by enzymatic electrophoresis.

Determination of ploidy levels

The ploidy levels of the 304 samples were assessed by DAPI-flow cytometry method (FCM). The samples were prepared according to De Laat & Blaas (1984) and De Laat & al. (1987). DAPI staines DNA only partially, so the absolute quantity of DNA is not measured. However, using DAPI with closely related taxa allows a relative measure of ploidy level, with often a better resolution than with intercaling dyes (Ulrich & al. 1988). A sample of chicken red blood cells (CRBC) served as internal standard for each analysed specimen, minimizing erroneous results. For about 10 plants, chromosomes were counted before the FMC analysis, according to the cell spreading technique of Pijnacker & Ferwerda (1984), providing references to interpret the first results. Later, chromosome counts were extended to 54 plants, chosen specifically from the extremes of the variation intervals of the ploidy levels obtained for each species. So, these counts were used as double checks.

Study of embryo sacs

A preliminary study of embyo sac development of 4 species of section *Brevivalvula* was made. Inflorescences at early flowering stage were collected and fixed in formalin-acetic acid-alcohol (FAA). Ovaries were embedded in paraffin, sectioned by microtome, stained in safranin-fastgreen and finally studied by conventional optic microscopy.

Estimation of isozymic variability

After the ploidy levels were obtained, isozyme polymorphism was determined with starch gel electrophoresis of the 73 *P. subangustum* and 105 *P. polystachion* plants (genotypes) of the sample, all newly sown from the collected seed lot. 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); following the techniques described by Wendel & Weeden (1989). The white part of young leaves were used in all analyses. The putative loci with their allelic variations were determined for each zymogram; only 1 locus could be interpreted per system. For each locus a different allelic combination was coded by a letter, so for each plant its genotype at all the loci was characterized by a 5 letter code.

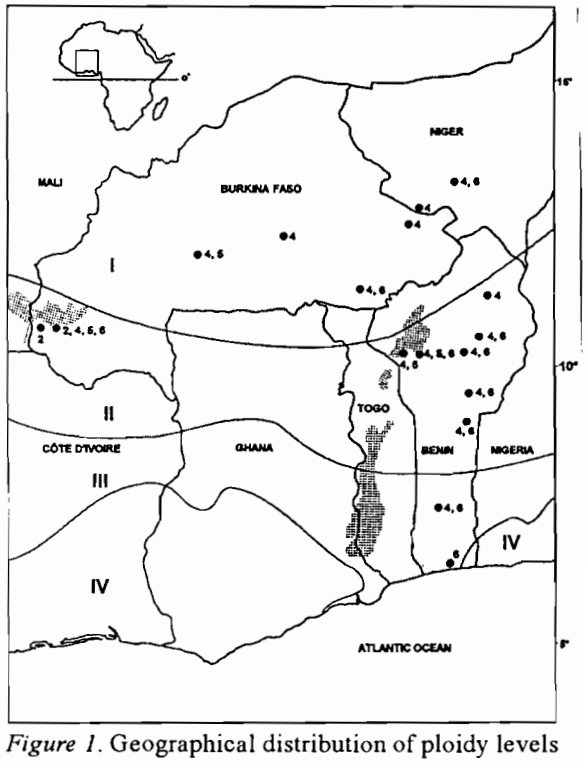

Figure 1. Geographical distribution of ploidy levels of section *Brevivalvula*. The numbers indicate the ploidy levels observed at each collection site marked by a black spot. The stippled pattern represent areas of relief.

Results

Geographical distribution of species

All species from sect. *Brevivalvula* were sampled except *P. atrichum*, which was observed at low densities at a few locations in

Table 1. Distribution of the samples per species, ploidy level and vegetation zone.

Taxa	Zone	4 X	6 X	5 X	2 X	total
P. pedicellatum	I	53	7	3		63
	II	27	6	1		34
P. hordeoides	I	3	1			4
	II	7				7
P. polystachion	I	26				26
	II	33	11	2	19	65
	III	2	12			14
P. subangustum	I	13				13
	II	33	6			48
	III	11	1			12
P. setosum	II		6		9	6
	III		12			12
Brevivalvula	I	95	8	3		106
	II	100	29	3	28	160
	III	13	25			38
total		208	62	6	28	304

Burkina Faso, but was not sampled because its seeds were not mature. Populations of the *Brevivalvula* species were mostly encountered in anthropic sites, around villages, roadsides and harvested fields or fallow land, but scattered populations occurred in the forest savannah zone, away from villages and cultivation. The species collected are distributed over large vegetation zones, defined by White (1986) as follows (Fig. 1) 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. Isohyets run more or less parallel to latitudes till isohyet 800 mm (Niger and most of Burkina Faso), increasing to 1200 mm towards the coast of Benin, with a circular isohyet of 1300 mm over the Atakora hills in zone II (Le Barbé & al. 1993).

Variability of ploidy levels

Among the 54 plants for which the ploidy level has been assessed by counting, only one individual does not have the right ploidy level estimated by FCM (4x instead of 6x), for the other 53 plants the estimated ploidy level corresponds with the chromosome counts. Consequently, four putative ploidy levels have been attributed to the remaining 250 samples analysed by FCM : diploid (2x), tetraploid (4x), pentaploid (5x), and hexaploid (6x). No aneuploids were observed. The coefficient of variation of samples studied by FCM varies between 2% and 9%, with 90% between 2% and 6%.

The ploidy levels are distributed unevenly over the species (**Tab. 1**) :

- diploids (9,2%) are only found in *P. polystachion* and *P. subangustum*. It the first time that diploids are found in these 2 annual species.
- tetraploids (68,4%) are dominant and are found in all species, except *P. setosum*.
- pentaploids (2,0%) are only observed in *P. polystachion* and *P. pedicellatum* in low numbers.
- hexaploids (20,4%) are found in all 5 species and areb the only level found in the sample *of P. setosum*.

Species and ploidy level distribution over the vegetation zones :

There is a gradual segregation of the species over the 3 vegetation zones. *P. pedicellatum* and *P. hordeoides* are only observed in zone I and II, *P. setosum*, the perennial species, only in zone II and III, while the other two species, *P. polystachion* and *P. subangustum* are present in all three vegetation zones. In vegetation zone II all four ploidy levels are present. In zone I no diploids are observed while in zone III neither diploids nor pentaploids are observed.

Intraspecific variation of ploidy levels :

Pennisetum pedicellatum shows three euploidy levels : 4x, 5x and 6x, each occurring in vegetation zone I and II. Tetraploids are dominant, with less hexaploids and only a few pentaploids.

Pennisetum hordeoides shows two euploid levels : 4x and 6x, the hexaploid occurring only once, in zone I. The tetraploids are present both in zone I and II.

Pennisetum polystachion is observed in the whole sampled area and shows the largest variation of ploidy levels : 2x, 4x, 5x, and 6x. Tetraploids are present in all three the zones, but they form the only group in zone I, and are the dominant group in zone II. Hexaploids are present in zone II and III and are dominant in zone III. Diploids and pentaploids only occurr in zone II, in the Banfora area in Burkina Faso.

Pennisetum subangustum shows three euploidy levels : 2x, 4x, and 6x, and follows the same geographical pattern as *P. polystachion*, with the exception of the hexaploids, which are dominant in zone II, not in zone III.

Pennisetum setosum occurs only in zone II and III and is characterized by only one ploidy level : 6x. The frequency of this species increases towards the south of Benin till the coast. In the northern part of zone II seeds were not ripe and could not be harvested.

Preliminary study of embryo sacs

Of five plants the embryo sacs were observed. Four plants (one *P. pedicellatum*, one *P. hordeoides* and two *P. polystachion*) are tetraploids (2n = 36) and have an apomictic reproduction system. One plant (*P. subangustum*) is diploid (2n = 18) and shows strictly sexual reproduction.

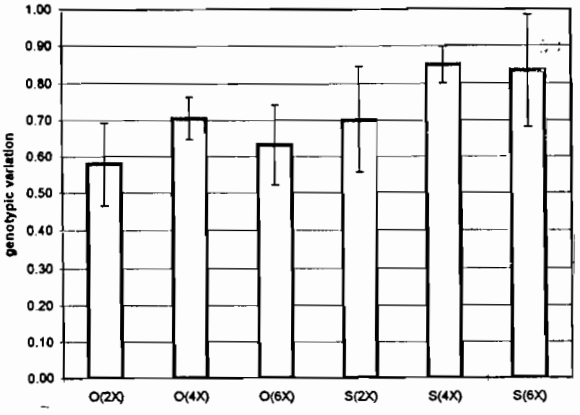

Figure 2. Genotypic variation of *P. polystachion* and *P. subangustum* in relation to their ploid levels.

***Isozyme variability in* P. polystachion *and* P. subangustum**

For each of the 5 enzymatic systems (ENP, PGD, PGM, IDH, PGI) one putative locus is interpreted. The loci used to characterize the genetic variability in *P. polystachion* and *P. subangustum* according their ploidy level, are polymorphic with a total of 25 alleles :

- ENP is a monomeric enzyme, 4 isozymes correspond to the allelic variations at 1 locus, the heterozygote phenotype is expressed by 2 bands for a diploid, 9 allelic combinations are observed in the sample.
- IDH is a dimeric enzyme, 6 isozymes correspond to the allelic variations at 1 locus, the heterozygote phenotype is expressed by 3 bands for a diploid, 6 allelic combinations are observed in the sample.
- PGD is a dimeric enzyme, 4 isozymes correspond to the allelic variations at 1 locus, the heterozygote phenotype is expressed by 3 bands for a diploid, 3 allelic combinations are observed in the sample.

- PGI is a dimeric enzyme, 7 isozymes correspond to the allelic variations at 1 locus, the heterozygote phenotype is expressed by 3 bands for a diploid, 11 allelic combinations are observed in the sample.

- PGM is a monomeric enzyme, 4 isozymes correspond to the allelic variations at 1 locus, the heterozygote phenotype is expressed by 2 bands for a diploid, 10 allelic combinations are observed in the sample.

The genotypic variability expressed by the proportion of different genotypes in each taxon is illustrated in figure 2 (the pentaploid *P. polystachion* plants could not be analysed statistically because the numbers are too low). The genotypic variability ranges between 0.58 for the diploid *P. polystachion* to 0.85 for the tetraploid *P. subangustum*, but is not significantly different among the 6 taxa.

Discussion

Past studies show that apomictic species are usually perennial and are often associated with interspecific hybridisation and polyploidy (Knox 1967; Asker 1980; Savidan 1982). The present study is a particular case because the section *Brevivalvula* shows four euploidy levels (x = 18, 36, 45 and 54) and a high level of apomixis (Chatterji & Pillai 1970; Sisodia & Raut 1980; Birari 1981; Kalyane & Chatterji 1981) primarily in annual species, with the exception of *P. setosum* , which is a perennial. Only one reference mentioned diploidy in section *Brevivalvula*, for one individual in the species *P. hordeoides* (Khosla & Mehra 1973). We have found diploids in two annual species, *P. polystachion* and *P. subangustum*, in a restricted area near Banfora, Burkina Faso, and a later prospection of the area south of Banfora, including the whole of Côte d'Ivoire, showed no continuation of this population.

The hypothesis that apomixis is a dead end in evolution has been discarded because of the discovery that most apomictic taxa are facultative apomicts and rare sexual populations permit to enhance the genetic diversity (Bashaw 1980). Moreover, sexuality may allow fertilisation of egg cells, both reduced and unreduced, allowing hybridization and further polyploidization between apomictic taxa. Variable progeny of supposed obligate apomicts has been demonstrated in *Taraxacum* (Lyman & Ellstrand 1984), some members of the subfamily *Malioideae* of the family *Rosaceae* (Campbell & Dickinson 1990), both from the temperate zone, and *Panicum maximum* (Savidan 1982), from the tropical zone. Assienan & Noirot (1995) found recently that isozyme polymorphism in the agamic complex of the *Maximae* is considerable, and thus does not lead to a reduction in diversity, if compared to sexual taxa.

Polyploidy balances possible negative effects of apomixis, providing a buffer against lethal mutations, and fixates enzyme diversity, while the residual sexuality releases the genetic diversity. As polyploid apomicts are often allopolyploid, they tend to be highly heterozygous. Jauhar (1981) concludes on the basis of multivalent frequency observed by Hrishi (1952), Pantulu (1969), Rangaswamy (1972), and Sisodia (1970) that *P. pedicellatum*, *P. polystachion*, and *P. subangustum* are of alloploid origin. The *Brevivalvula* section is characterized by polyploid populations in contact with rare diploid populations and by high genotypic variability in polyploid, apomictic populations, as shown in isozyme analysis of *P. polystachion* and *P. subangustum*. This situation agrees with a mechanism of the diploid-tetraploid-haploid cycle type, described in the *Bothriochloa-Dichanthium* complex (De Wet 1968; De Wet 1971) and later in *Panicum maximum* (Savidan 1982). Once tetraploids are established, pentaploids and hexaploids could be formed through further polyploidization

Since polyploidy is intensified by interspecific hybridisation, it is among such hybrid complexes where apomixis is commonly encountered. Once established, apomixis contributes towards higher fitness by fixing and reproducing advantageous genotypes (De Wet & Stalker 1974). The more is known of polyploidy combined with apomixis and sexuality in natural populations, the better it can be used in experimental research as a means of improving cultivated plants in general and, in our case, *P. glaucum*, pearl millet.

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