Pl. Syst. Evol. 198: 89–100 (1995)

Plant Systematics and Evolution

Variation and geographical distribution of ploidy levels in *Pennisetum* section *Brevivalvula* (*Poaceae*) in Burkina Faso, Benin and southern Niger

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

Received November 8, 1994; in revised version April 19, 1995

Key words: *Poaceae, Pennisetum* sect. *Brevivalvula*. – Polyploidy, apomixis, flow cytometry.

Abstract: *Pennisetum* sect. *Brevivalvula* is a species complex characterized by polyploidy and apomixis. Ploidy level was 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 Africa. Diploid populations of *P. polystachion* and *P. subangustum* were found in the Banfora area, in Burkina Faso.

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 an apomictic reproduction system, which could be used to produce an apomictic pearl millet [*P. glaucum* (L.) R. Br.] by experimental introgression of new genes (Hanna & Bashaw 1987, Savidan & Dujardin 1992).

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 specimens, Brunken (1979 b) considered *P. polystachion, P. setosum,* and *P. atrichum* as one species, called *P. polystachion,* divided into three subspecies, *P. polystachion* subsp. *polystachion*, *P. polystachion* subsp. *setosum* (Sw.) Brunken and *P. polystachion* subsp.

atrichum (Stapf & Hubb.) Brunken. *Pennisetum 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* (Chowdhury & 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 (1979 a) 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 (1979 b) 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. Sect. *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 analyse 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 analysed 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 Doležel (1991) analyses 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 299, 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, 1 mM 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 tissues 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). The 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 & 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 analysed specimen. Thus, the DNA ratio between the sample and the standard could be measured apart from a possible derivation in relation to a fixed 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 FMC 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

Table 1. Number of plants per ploidy level, according to species *Pennisetum 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	8°55.83′ N	2°35.07′ E				6
E	III	43	7°23.49′ N	2°04.50′ E				3
E	III	48	6°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	9°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	9°27.28′ N	2°37.55′ E		5		3
O	II	38	8°55.83′ N	2°35.07′ E		4		3
O	III	43	7°23.49′ N	2°04.50′ E		2		1
0	III	48	6°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	8°55.83′ N	2°35.07′ E		1		6
S	III	43	7°23.49′ N	2°04.50′ E		11		1

of ratios obtained through FCM would be covered, in particular the extreme values of each interval.

Preliminary study of embryo sacs. Inflorescences at early flowering 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, follow 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 sampled within sect. *Brevivalvula* has its own geographical distribution through four large vegetation zones defined by White (1986) as follows (Figs. 1, 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 [2.41–2.98].

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 analysed only by FCM, we attributed the putative ploidy level agreeing with the interval where a particular ratio was found.

Interspecific variation in ploidy levels. *Pennisetum 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* (χ^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* (χ^2 = 10,45, d.f. = 1).

Table 2. Ploidy levels (2n) obtained through chromosome counting in the species *Pennisetum setosum* (E), *P. hordeoides* (H), *P. polystachion* (O), *P. pedicellatum*, 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	1.7	4x	8
H	30	1.72	4x	5
H	23	2.68	6x	5
O	19	0.8	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	1.7	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	2.5	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	2.9	6x	4
E	43	2.98	6x	2
P	30	1.5	4x	6
P	9	1.6	4x	5
P	3	1.7	4x	4
P	31	1.76	4x	5
P	31	1.8	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	2.5	6x	3
P	23	2.68	6x	8
S	19	0.77	2x	5
S	19	0.8	2x	5
S	21	0.82	2x	7
S	21	0.88	2x	6
S	43	1.62	4x	4
S	26	1.7	4x	8
S	29	1.73	4x	5

Table 2 (continued)

Species	Site	Ratio	2n	Cell
S	27	1.8	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

Pennisetum 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:

Pennisetum pedicellatum (Fig. 1) was observed only in the north of the studied area. It shows three 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.

Pennisetum 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 analysed were all hexaploids.

Pennisetum 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.

Pennisetum 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 analysed plants were diploid, the others being tetraploid. The 14 plants originating from vegetation zone III are hexaploid 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.

Pennisetum subangustum (Fig. 2) follows the same geographical patterns as *P. polystachion* and shows three euploidy levels: 2x, 4x, 6x.

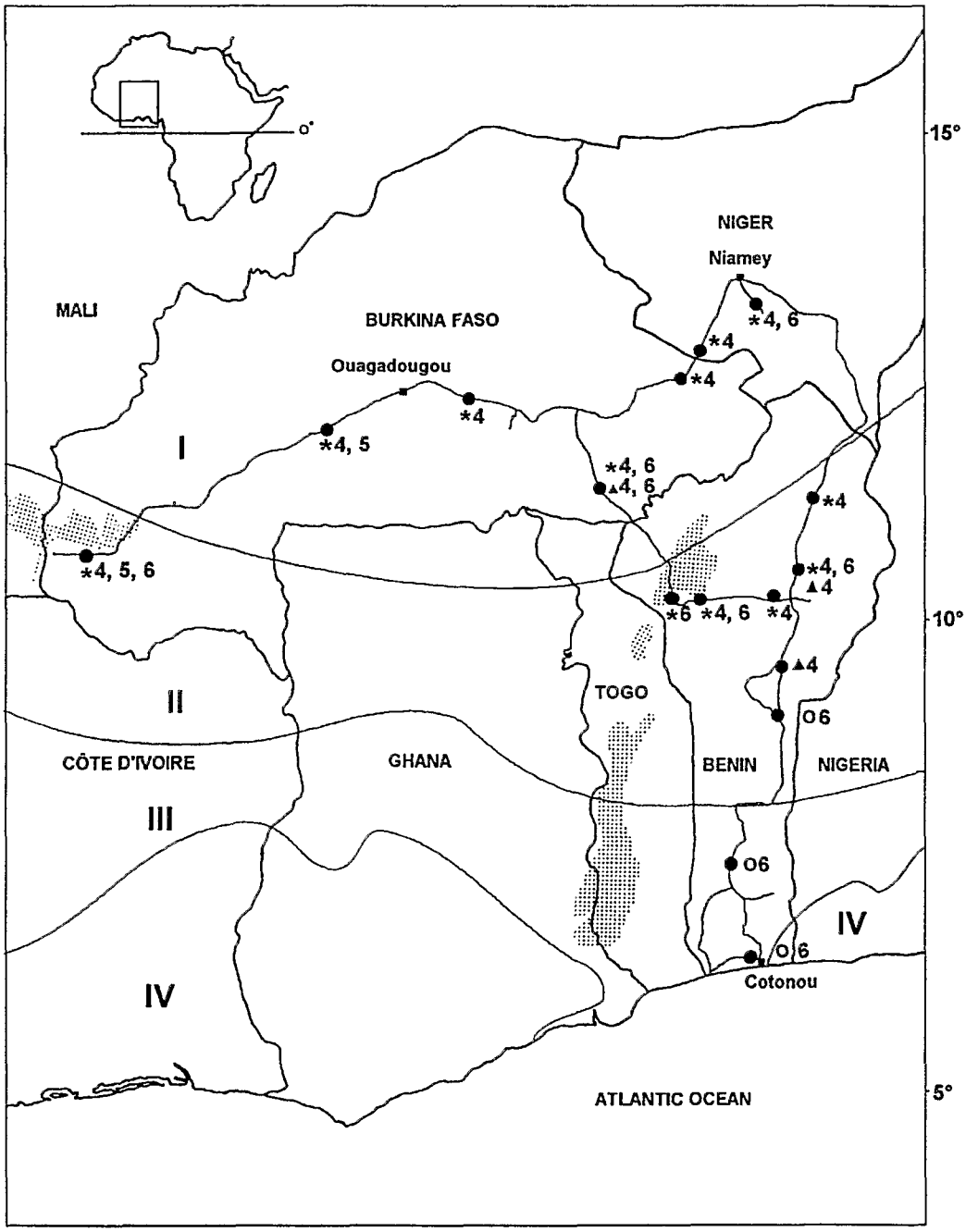

Fig. 1. Geographical distribution of ploidy levels in the species *Pennisetum pedicellatum* (*), *P. hordeoides* (▲) and *P. setosum* (○). The stippled pattern represent areas of relief

All analysed 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 analysed plants.

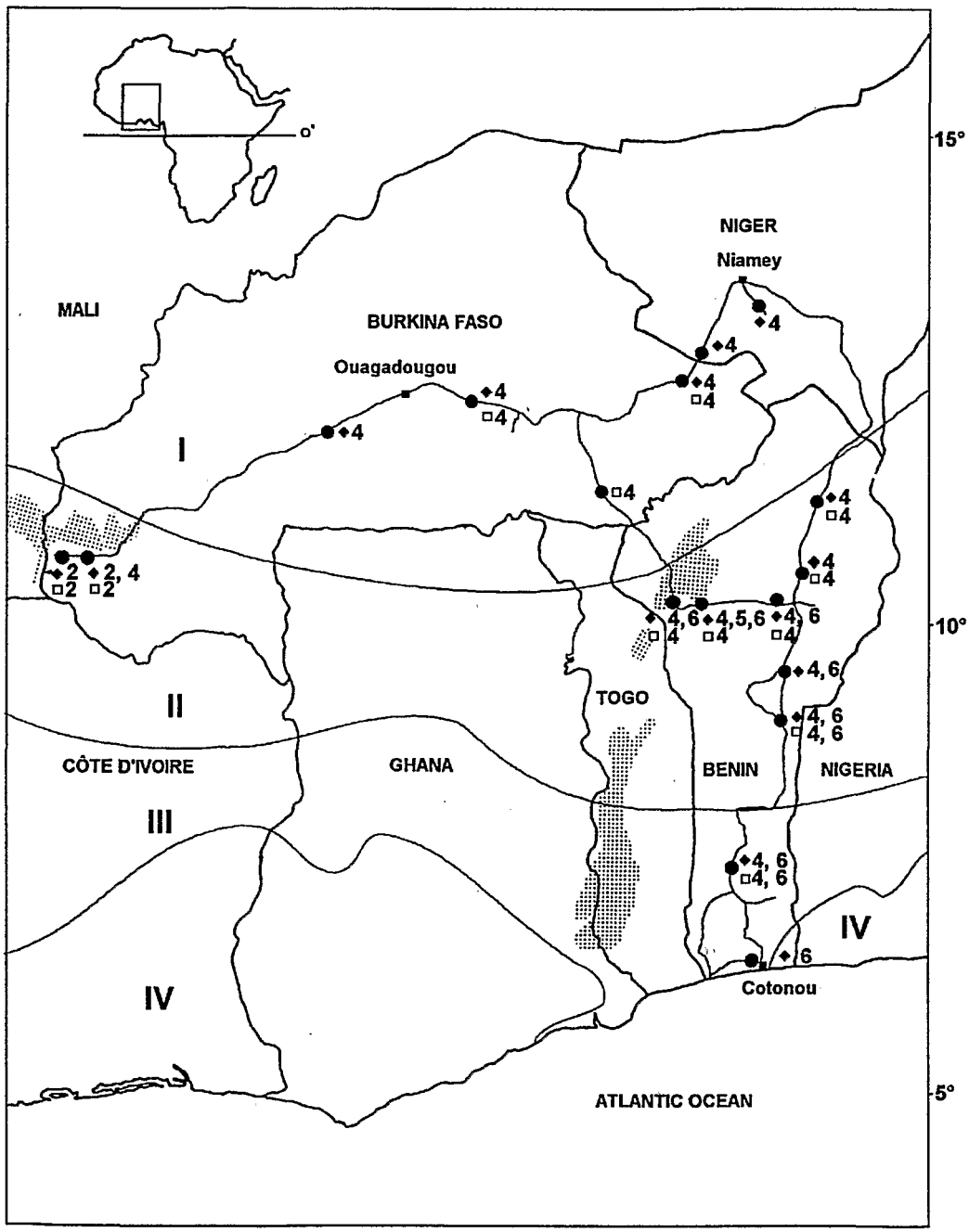

Fig. 2. Geographical distribution of ploidy levels in the species *Pennisetum polystachion* (◆) and *P. subangustum* (□). The stippled pattern represent areas of relief

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 sect. *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 level.

Apomicts are often allopolyploid 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 perennial and diploid like the parental species (Asker & Jerling 1992). The only perennial species in sect. *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 sect. *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 diploid populations with obligate sexuality 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 increase of the genome size and perennity

could be related to a higher annual 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 (1979 a) 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 adaptive 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* L. from Spain, for example, diploid populations grow in woodlands and tetraploid populations in open areas (LUMARET & al. 1987 a, b). Among adaptive strategies, apomixis and polyploidy often go together (DE WET 1971). 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.

We gratefully acknowledge valuable comments on the manuscript by Prof. L. J. G. VAN DER MAESEN, from the Wageningen Agricultural University (The Netherlands); Dr R. LUMARET from the CNRS (France); Dr G. BEZANÇON AND Dr S. HAMON from ORSTOM; Dr C. RENARD from ICRISAT (India); Ir. G. GEENEN, of the "Plant Cytometric Services" in Schijndel (The Netherlands), for the flow cytometry analyses; Prof. M. T. M. WILLEMSE, from the Wageningen Agricultural University (The Netherlands) for helping to interpretate the embryo sacs and Dr Y. SAVIDAN for his communicative enthusiasm. The research institutes "ICRISAT" and "ORSTOM" we thank for financing the collecting tours.

References

ASKER, S. E., JERLING, L., 1992: Apomixis in plants. – Florida: CRC Press.

BIERZYCHUDEK, P., 1985: Patterns in plant parthenogenesis. – Experientia **41**: 1255–1264.

BIRARI, S. P., 1981: Mechanisn of apomixis in *Pennisetum polystachion* SCHULT. – J. Maharashtra Agr. Univ. **6 (3)**: 208–221.

BOR, N. L., 1960: Grasses of Birma, Ceylon, India and Pakistan. – London: Pergamon Press.

BRUNKEN, J. N., 1979 a: Cytotaxonomy and evolution in *Pennisetum* section *Brevivalvula* (*Gramineae*) in tropical Africa. – Bot. J. Linn. Soc. **79**: 37–49.

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

CHOWDHURY, M. K. U., SMITH, R. L., 1988: Mitochondrial DNA variation in pearl millet and related species. – Theor. Appl. Genet. **76**: 25–32.

CLAYTON, W. D., 1972: *Gramineae*. 101. *Pennisetum*. – In HEPPER, F. N. (Ed.): Flora of West Tropical Africa (III), pp. 459–462. – London: Crown Agents.

DE LAAT, A. M. M., BLAAS, J., 1984: Flow cytometric characterisation and sorting of plant chromosomes. – Theor. Appl. Genet. **67**: 463–467.

– GÖDHE, W., VOGELZANG, M. J. D. C., 1987: Determination of ploidy of single plants and plant populations by flow cytometry. – Plant Breeding **99**: 303–307.

DE WET, J. M., 1971: Polyploidy and evolution in plants. – Taxon **20**: 29–35.

Doležel, J., 1991: Flow cytometry analysis of nuclear DNA content in higher plants. – Phytochem. Analysis **2**: 143–154.

Gupta, V. P., Minocha, J. L., 1980: Trends in genetical research on Pennisetums. – Ludhiana: Punjab Agricultural University.

Gustafsson, A., 1947: Apomixis in higher plants. II. The causal aspect of apomixis. – Lunds Univ. Arsskr. **43**: 71–178.

Hanna, W. W., Bashaw, E. C., 1987: Apomixis: its identification and use in plant breeding. – Crop Sci. **26**: 1136–1139.

Kalyane, V. L., Chatterji, A. K., 1981: Reproductive characteristics of *Pennisetum pedicellatum*. – Indian J. Genet. **41**: 384–388.

Khosla, P. K., Mehra, P. N., 1973: IOPB chromosome number reports. XLI. – Taxon **22**: 650–651.

Lagudah, E. S., Hanna, W. W., 1990: Patterns of variation for seed proteins in the *Pennisetum* gene pool. – J. Heredity **81**: 25–29.

Le Barbé, L., Alé, G., Millet, B., Texier, H., Borel, Y., Gualde, R., 1993: Les ressources en eaux superficielles de la République de Bénin. – Paris: Editions de l'ORSTOM, Collection Monographies Hydrologiques N° 11.

Lumaret, R., 1988: Adaptive strategies and ploidy levels. – Acta Oecologica/Oecol. Plant. **9**: 83–93.

– Barrientos, E., Guillerm, J. L., Jay, M., Fiasson, J. L., Ardouin, P., Delay, J., Ait Lhaj Loutfi, A., Izco, J., Amigo, J., 1987 a: Signification adaptive et évolutive de la polyploidie infraspécifique: cas de dactyles diploides et tetraploides de Galice (Espagne). – In Legay, J. M., (Ed.): Proceedings Coll. Nat. C.N.R.S. "Biologie des populations", pp. 129–133. – Lyon: Université Claude Bernard.

– Guillerm, J. L., Delay, J., Loutfi, A., Izco, J., Jay, M., 1987 b: Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). – Oecologia (Berlin) **73**: 436–446.

Marie, D., Brown, S. C., 1993: A cytometric exercise in plant DNA histograms, with 2C values for 70 species. – Biol. Cell **78**: 41–51.

Mayer, E., 1974: Populations, espèces et évolution. – Paris: Hermann.

Morel, R., 1992: Atlas agroclimatique des pays de la zone du CILSS. – Niamey: CILSS/PNUD/OMM.

Pijnacker, L. P., Ferwerda, M. A., 1984: Giemsa C-banding of potato chromosomes. – Canad. J. Genet. Cytol. **26**: 415–419.

Savidan, Y., Dujardin, M., 1992: Apomixie: la prochaine révolution verte? – La Recherche **241 (23)**: 326–334.

Skerman, P. J., Riveros, F., 1990: Tropical grasses. – Rome: FAO Pl. Production Protection Ser. **23**.

Stapf, O., Hubbard, C. E., 1934: *Pennisetum*. – In Prain, D., (Ed.): The flora of tropical Africa, pp. 954–1070. – Ashford: Reeve.

Ulrich, U., Fritz, B., Ulrich, W., 1988: Application of DNA fluorochromes for flow cytometric analysis of plant protoplasts. – Pl. Sci. **55**: 151–158.

White, F., 1986: La végétation de l'Afrique. – Paris: ORSTOM – UNESCO.

Yadav, M. S., Zadoo, S. N., Bhag Mal, Tripathi, S. N., 1980: Chromosomal races of *Pennisetum pedicellatum* in relation to their economic traits. – In Gupta, V. P., Minocha, J. L., (Eds): Trends in Genet. Res. Pennisetums, pp. 209–213. – Ludhiana: Punjab Agricultural University.

Addresses of the authors: J.-F. Renno, ORSTOM, Laboratoire de génétique, B.P. 11416, Niamey, Niger. – G. H. Schmelzer, DFPV - ORSTOM, Laboratoire de génétique, B. P. 11416, Niamey, Niger. – J. H. De Jong, Wageningen Agricultural University, Department of Genetics, Dreijenlaan 2, NL-6703 HA Wageningen, The Netherlands.

Accepted April 19, 1995 by F. Ehrendorfer