Euphytica **96:** 23–29, 1997.

Don Au
Pels

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

G.H. Schmelzer[1] & J.-F. Renno[2]
[1] *DPFV – ORSTOM, Genetic Laboratory, B.P. 11416, Niamey, Niger;* [2] *ORSTOM, Genetic Laboratory, B.P. 11416, Niamey, Niger*

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

Summary

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 hilly regions displayed a higher ploidy diversity than the others; diploid populations of the annual species *P. polystachion* and *P. subangustum* were found. Genotypic variation expressed by isozyme polymorphism did not show any significant difference 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 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 improvement of agronomic traits 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 often harbour one or sometimes more apomixis genes which can be transferred to cultivated varieties by crosses and backcrosses (Asker and 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, whereas the apomictic wild species is polyploid. Improvements in this type of research have been achieved 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 crosses, 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 similar research has been conducted 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 and Hanna, 1983), *P. squamulatum* (Hanna et al., 1993), *P. setaceum* (Hanna, 1979), *P. schweinfurthii* (Hanna and Dujardin, 1986). No interspecific hybrids were produced, in the samples available, between pearl millet

P. glaucum and either *P. ramosum*, *P. mezianum*, *P. macrourum*, *P. pedicellatum*, or *P. polystachion*. However, there was partial seed development in diploid pearl millet × *P. pedicellatum* and *P. polystachion* (Dujardin & Hanna, 1989).

The genus *Pennisetum* has been divided into 5 sections: *Gymnothrix*, *Eu-Pennisetum*, *Penicillaria*, *Heterostachya*, and *Brevivalvula* and consists of some 120–130 species worldwide, in tropical and warm regions, according to Stapf and Hubbard (1934). These authors cite 91 species in Tropical Africa, while Lebrun and 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 thought 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, 1971) 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 (1979a) and Jauhar (1981) have reviewed 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 et al. (1995), as well as enzymatic variations, in a sample collected from a part of the species distribution area, but through distinct ecological zones such as plains, hilly areas, and the coastal region.

Materials 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*. The species are differentiated by their life cycles, the number of spikelets per involucre ans whether they are sessile or not, the number of bristles of the involucre and whether these bristles are hairy or not (Stapf and Hubbard 1934; Bor 1960; Clayton 1972; Brunken 1979b).

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 Faso, 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 allows quick access to morphological variation but tends to overestimate the real polymorphism of populations, because rare genotypes are chosen at the expense of possibly predominant genotypes in a population. For each seed lot sampled from a parental plant *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 and Blaas (1984) and De Laat et al. (1987). DAPI stains 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 et al., 1988). A sample of chicken red blood cells (CRBC) served as internal standard for each analysed specimen, minimizing artefactual results. For each specimen the histogram of the DNA quantities estimated by DAPI for each nucleus is obtained. For a histogram the position of its peak compared to the CRBR peak is proportional to the quantity of DNA, while the coefficient of variation (CV) indicates the limit of resolution. For about 10 plants, chromosomes were counted before the FMC analysis, according to the cell spreading technique of Pijnacker and 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 embryo 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. Sexual ovaries showed 8-nucleate embryo sacs, while the other ovaries showed unreduced, 4-nucleate, aposporous embryo sacs.

Estimation of isozymic variation

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. The white part of young leaves were used in all analyses. 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 and the zymogram interpretation described by Wendel and Weeden (1989). The putative loci with their allelic variations were

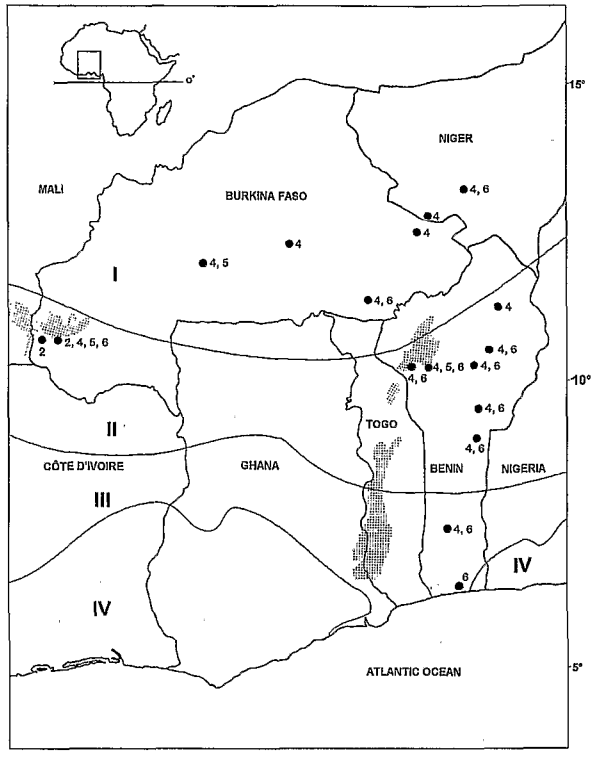

Figure 1. Geographical distribution of ploidy levels of section *Brevivalvula*. The numbers indicate the ploidy levels observed at each collection site marked by a black spot. The stippled pattern represents hilly areas.

deducted 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.

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 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 ploidy levels of the species collected are distributed over large vegetation zones

(Figure 1), defined by White (1986) as follows: 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 Bein, with a circular isohyet of 1300 mm over the Atakora hills in zone II (Le Barbé et al., 1993).

Variation of ploidy levels

Among the 54 plants for which the ploidy level was assessed by counting, only one individual did not have the right ploidy level estimated by FCM (4x instead of 6x); for the other 53 plants the estimated ploidy level corresponded with the chromosome counts. Consequently, four putative ploidy levels were 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 varied between 2% and 9%, with 90% between 2% and 6%.

The ploidy levels are distributed unevenly over the species (Table 1):

- diploids (9.2%) are only found in *P. polystachion* and *P. subangustum*. It is 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 observed only in *P. polystachion* and *P. pedicellatum* in low numbers.
- hexaploids (20.4%) are found in all 5 species and are the only level found in the sample of *P. setosum*.

Species and ploidy level distribution over the vegetation zones

There is a segregation of the species over the 3 vegetation zones. *P. pedicellatum* and *P. hordeoides* are only observed in zones I and II; *P. setosum*, the perennial species, only in zones 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.

Interspecific variation of ploidy levels

Pennisetum pedicellatum shows three euploidy levels: 4x, 5x and 6x, each occurring in vegetation zone I and zone 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 zones, but they are exclusive in zone I, and are predominant in zone II. Hexaploids are present in zone II and III and are predominant in zone III. Diploids and pentaploids only occur in zone II, in the Banfora area of 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 fewer in zone III.

Pennisetum setosum occurs only in zone II and III and is characterized by a single 6x ploidy level. 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

The embryo sacs were observed for five plants. Four plants (one *P. pedicellatum*, one *P. hordeoides* and two *P. polystachion*) were tetraploids (2n = 36) and had an apomictic reproduction system. One plant (*P. subangustum*) was diploid (2n = 18) and showed strictly sexual reproduction.

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

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

- ENP is a monomeric enzyme; the heterozygote phenotype is expressed by 2 bands for a diploid, 4 isozymes corresponded to the allelic variations at 1 locus, 9 distinct genotypes were observed in the sample.
- IDH is a dimeric enzyme; the heterozygote phenotype is expressed by 3 bands for a diploid, 6

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

Taxa	Zone	4x	6x	5x	2x	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		9	48
	III	11	1			12
P. setosum	II		6			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

homodimeric isozymes corresponded to the allelic variations at 1 locus, 6 distinct genotypes were observed in the sample.

- PGD is a dimeric enzyme; the heterozygote phenotype is expressed by 3 bands for a diploid, 4 homodimeric isozymes corresponded to the allelic variations at 1 locus, 3 allelic combinations are observed in the sample.
- PGI is a dimeric enzyme; the heterozygote phenotype is expressed by 3 bands for a diploid, 7 homodimeric isozymes corresponded to the allelic variations at 1 locus, 11 allelic combinations are observed in the sample.
- PGM is a monomeric enzyme; the heterozygote phenotype is expressed by 2 bands for a diploid, 4 isozymes corresponded to the allelic variations at 1 locus, 10 allelic combinations are observed in the sample.

The genotypic variation of the chromosomal races of the two species is expressed by the frequency of different genotypes in all the samples analysed, as illustrated in Figure 2. For example, a frequency of 0.45 for a chromosomal race would indicate that of 100 plants analysed, 45 have each a different 5 letter code, and the 55 others have a same code. The pentaploid *P. polystachion* plants could not be analysed statistically because the numbers are too low. The genotypic variation ranged between 0.58 for the diploid *P. polystachion* to 0.85 for the tetraploid *P. subangustum*, but was not significantly different among the six chromosomal races of these two species.

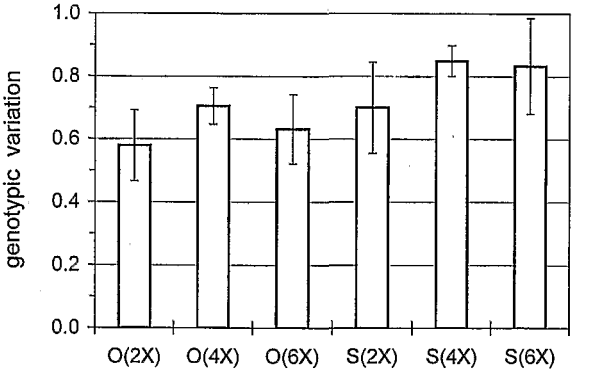

Figure 2. Genotypic variation of the chromosomal races of *P. polystachion* (O) and *P. subangustum* (S) in relation to their ploidy levels.

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 (2n = 18, 36, 45 and 54) and a high lev-

el of apomixis distributed primarily in annual species, with the exception of *P. setosum*, which is a perennial (Chatterji & Pillai, 1970; Sisodia & Raut, 1980; Birari, 1981; Kalyane & Chatterji, 1981). Only one reference so far mentioned diploidy in section *Brevivalvula*, for one individual in the species *P. hordeoides* (Khosla & Mehra, 1973). We found diploids in two annual species, namely *P. polystachion* and *P. subangustum*, in a restricted area near Banfora, Burkina Faso, and a recent 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 that sexual populations, even rare, 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, as well as *Panicum maximum* (Savidan, 1982), from the tropical zone. Assienan and Noirot (1995) recently found that isozyme polymorphism in the agamic complex of the *Maximae* is very high, and thus contradicts a reduction in diversity, if compared to sexual taxa.

Polyploidy balances possible negative effects of apomixis, providing a buffer against lethal mutations, and fixes genotypic diversity, while the residual sexuality releases it. As polyploid apomicts are often allopolyploid, they tend to be highly heterozygous. Jauhar (1981) concluded 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 variation 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, 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 that 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.

Acknowledgements

We express our gratitude to Ir. G. Geenen, of the 'Plant Cytometric Services' in Schijndel (The Netherlands), for the flow cytometry analyses, Dr. J.H. de Jong and Prof. M.T.M. Willemse, both from the Wageningen Agricultural University (The Netherlands), the former for the chromosome counts, the greenhouse facilities and last but not least his valuable comments, and the latter for his help preparing and interpreting the embryo sacs. We thank the research institutes ORSTOM financing the research and ICRISAT for financing the collecting trips.

References

Asker, S.E., 1980. Gametophytic apomixis: elements and genetic regulation. Hereditas 93: 277–293.

Asker, S.E. & L. Jerling, 1992. Apomixis in plants. CRC Press, Florida.

Assienan, B. & M. Noirot, 1995. Isozyme polymorphism and organization of the agamic complex of the *Maximae* (*Panicum maximum* Jacq., *P. infestum* Anders, and *P. trichocladum* K. Schum.) in Tanzania. Theor Appl Genet 91: 672–680.

Bashaw, E.C., 1980. Apomixis and its application in crop improvement. In: W.R. Fehr & H.H. Hadley (Eds). Hybridisation of Crop Plants, pp. 45–63. American Society of Agronomy Press, Madison, WI.

Birari, S.P., 1981. Mechanism 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.

Brown, W.V. & W.H.P. Emery, 1958. Apomixis in the *Gramineae: Panicoideae*. Am J Bot 45: 253–263.

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

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

Burton, C.W., 1944. Hybrids between Napier grass and cat-tail millet. J Hered 35: 226–232.

Campbell, C.S. & T.A. Dickinson, 1990. Apomixis, patterns of morphological variation, and species concept in subfamily *Malioideae* (*Rosaceae*). Syst Bot 15: 124–135.

Chatterji, A.K. & G.K. Pillai, 1970. Apomixis in *Pennisetum pedicellatum*. Trin Sci Cult 36: 667–669.

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

De Laat, A.M.M. & J. Blaas, 1984. Flow cytometric characterisation and sorting of plant chromosomes. Theor Appl Genet 67: 463–467.

De Laat, A.M.M., W. Gödhe & M.J.D.C. Vogelzang, 1987. Determination of ploidy of single plants and plant populations by flow cytometry. Plant Breeding 99: 303–307.

De Wet, J.M.J., 1968. Diploid-tetraploid-haploid cycles and the origin of variability in *Dichanthium* agamospecies. Evolution 22: 394–397.

De Wet, J.M.J., 1971. Polyploidy and evolution in plants. Taxon 20: 29–35.

De Wet, J.M.J. & H.T. Stalker, 1974. Gametophytic apomixis and evolution in plants. Taxon 23: 689–697.

Dujardin, M. & W.W. Hanna, 1983. Apomictic and sexual pearl millet × *Pennisetum squamulatum* hybrids. J Hered 74: 277–279.

Dujardin, M. & W.W. Hanna, 1989. Crossability of pearl millet with wild *Pennisetum* species. Crop Sci 29: 77–80.

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

Hanna, W.W., 1979. Interspecific hybrids between pearl millet and fountaingrass. J Hered 70: 425–427.

Hanna, W.W. & M. Dujardin, 1986. Cytogenetics of *Pennisetum schweinfurthii* Pilger and its hybrids with pearl millet. Crop Sci 26: 449–453.

Hanna, W.W., M. Dujardin, P. Ozias-Akins, E. Lubbers & L. Arthur, 1993. Reproduction, cytology and fertility of pearl millet × *Pennisetum squamulatum* BC4 plants. J Hered 84 (3): 213–216.

Harlan, J.R. & J.M.J. De Wet, 1971. Towards a rational classification of cultivated plants. Taxon 20 (4): 509–517.

Hrishi, N.J., 1952. Studies on the cytogenetics of six species of Pennisetum and their comparative morphology and anatomy. Genetica 26: 280–356.

Jauhar, P.P., 1981. Cytogenetics and breeding of pearl millet and related species. Alan R. Liss, Inc., NY.

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

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

Knox, R.B., 1967. Apomixis: seasonal and population differences in a grass. Science 157: 325–326.

Le Barbé, L., G. Alé, B. Millet, H. Texier, Y. Borel & R. Gualde, 1993. Les ressources en eaux superficielles de la République du Bénin. Editions de l'ORSTOM, Collection Monographies Hydrologiques No 11, Paris.

Lebrun, J.-P. & A.L. Stork, 1995. Enumération des plantes à fleurs d'Afrique tropicale. Vol. III – Monocotylédones: *Limnocharitaceae à Poaceae*. Conservatoire et Jardin botaniques de la ville de Genève, Genève.

Lyman, J.C. & N.C. Ellstrand, 1984. Clonal diversity in *Taraxacum officinale* (*Compositae*), an apomict. Heredity 53 (1): 1–10.

Pantulu, J.V., 1969. Meiosis in two polymorphic species of *Pennisetum*. Curr Sci 38: 122–123.

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

Rangaswamy, S.R.S., 1972. Cytological studies on diploid and polyploid taxa of the genus *Pennisetum* Rich. Genetica 43: 257–273.

Renno, J.-F., G.H. Schmelzer & J.H. De Jong, 1995. Variation and geographical distribution of ploidy levels in *Pennisetum* section *Brevivalvula* (*Poaceae*) in Burkina Faso, Benin and southern Niger. Pl Syst Evol 198 (11-2): 89–100.

Savidan, Y., 1982. Nature et hérédité de l'apomixie. Travaux et documents de l'ORSTOM.

Savidan, Y., 1995. Les promesses de l'apomixie. ORSTOM Actualités 47: 2–7.

Sisodia, K.P.S., 1970. Cytological studies on some species in genus *Pennisetum*. Theor Appl Genet 40: 26–31.

Sisodia, K.P.S. & R.N. Raut, 1980. Meiotic behaviour and fertility of hexaploid *Pennisetum pedicellatum* Trin. In: V.P. Gupta & J.L. Minocha (Eds). Trends in Genetic Resources of Pennisetums, pp. 215–216. Punjab Agricultural University, Ludhiana.

Skerman, P.J. & F. Riveros, 1990. Tropical grasses. FAO Pl Protection Ser 23, Rome.

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

Tjitrosoedirdjo, S.S., 1990. *Pennisetum polystachion* (L.) Schult. Weed Info Sheet 3, 2 p., The Southeast Asian Weed Information Centre (SEAWIC), Indonesia.

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

Wendel, J.F. & N.F. Weeden, 1989. Visualization and interpretation of plant isozymes. In: D.E. Soltis & P.S. Soltis (Eds). Isozymes in Plant Biology, pp. 5–45. Chapman and Hall, London.

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