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Breeding *Panicum maximum* in Brazil. 1. Genetic resources, modes of reproduction and breeding procedures

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Summary

Apomixis is widely distributed among tropical forage grasses, and has long been merely regarded as an impediment to breeding. *Panicum maximum* is presented as the first opportunity for Brazilian geneticists to develop and test original breeding schemes adapted to an apomictic species.

A large and representative germplasm of *P. maximum* has been introduced and is currently being evaluated. Basic knowledges on biology and reproduction are also available, which demonstrate an easy manipulation of apomixis and sexuality. Several limiting traits have already been detected during evaluation, which justify breeding attempts. An ideal scheme is given to transfer new qualities to already selected varieties.

Introduction

Experience in breeding tropical forage grasses is still extremely limited. For most genera, plant introduction programs and subsequent agronomical evaluations need to be completed. Very few plant collections can at present time be considered representative of the natural variability, and as such be used as a good basis for a plant breeding program. Another strong limitation comes from the reproductive behavior. Success in breeding tropical forage grasses has been mainly limited to crosspollinating species, as exemplified by Burton's famous *Cynodon* varieties (Burton, 1947, 1972; Burton & Monson, 1978). Meanwhile, most cultivated tropical forage grasses are facultative or obligate apomicts (Brown & Emery, 1958; Bashaw, 1975). The only well known success which concerns an apomict, is Bashaw's breeding program on *Cenchrus ciliaris* (Taliaferro & Bashaw, 1966; Bashaw, 1968, 1980).

In *Panicum maximum*, breeding limitations due to inadequate genetic resources and lack of knowledge on how to manipulate apomixis, have now also been overstepped.

P. maximum Jacq. is one of the most important forage grass species cultivated in Brazil, where it is known as 'Capim Colonião'. Numerous Brazilian observers have mistakenly called it native to Brazil, as a consequence of its extraordinary ability to adapt outside its natural distribution. According to Parsons (1972), however, guineagrass entered Latin America at the end of the seventeenth century. Chase (1944) claimed that it was probably introduced as bedding on slave ships, establishing itself wherever these vessels unloaded. As slaves were caught along the western coast of Africa, while the species diversity was mainly restricted to East Africa (Combes & Pernes, 1970), the plants introduced at this time only exhibited the reduced polymorphism of marginal populations, an extremely low variability being therefore available for Brazilian farmers. One can postulate that the same has occured to other cultivated forage grasses, as most *Brachiaria* species, *Melinis minutiflora*, and *Cenchrus ciliaris*. Furthermore, as all these species reproduce through apomixis, roughly defined as asexual reproduction by means of seed (Nogler, 1984), no possibility of genetic recombination was offered to these grasses in their new Latin American home. As such, the present variation within Brazilian cultivars of *P. maximum* is still very low. Although a few introductions were made since the beginning of this century, they were limited to Australian cultivars, and never spread due to low adaptability and/or low seed production. Within the 'Colonião' morphological type, several cultivars have afterwards been identified, which presumably are nothing more than the consequence of guineagrass ability to respond to environmental variations.

For Brazilian agronomists, new introductions from East Africa were a must, and this was made possible in 1982 through the EMBRAPA-ORSTOM agreement. More than 400 apomictic accessions of *P. maximum* were introduced, along with several hundred seeds from sexual genotypes. An agronomical evaluation program was started (Savidan et al., 1985). Similar evaluations of the same material, are now being conducted in Colombia by Centro Internacional de Agricultura Tropical (CIAT), in Mexico by the Colegio de Postgraduados, and in Cuba by the Indio Hatuey Research Center. Another excellent evaluation program is carried out in Japan (Nakajima et al., 1978), although based on a more reduced variation.

Although breeding schemes have already been proposed for the species (Pernes et al., 1975; Smith, 1975), very little has been done in this way, except recently for seed production improvement (Noirot, 1985, Noirot et al., 1986). The *Panicum*-germplasm represents the first opportunity for Brazilian geneticists to breed an apomictic grass species, and since:

1. a large and representative germplasm has been collected,
2. basic knowledge on biology and reproduction is available,
3. limiting factors exist within the natural variation, such as very infrequent qualitative traits or poorly combined quantitative traits, we propose to follow up the agronomical evaluation with an original plant breeding scheme, as presented below.

Panicum maximum has been extensively collected by French and Japanese geneticists (Combes & Pernes, 1970; Nakajima et al., 1978), and a large germplasm is now available for breeding. This germplasm can be considered representative of the natural variation and further collects are not necessary.

In relation to the second point, and as most tropical forage grasses are apomictic, basic studies on the nature and inheritance of apomixis are strongly needed before any breeding attempt is made. This basic information is generally unavailable. *Panicum*, however, is an exception to that rule, as will be seen in the next section.

The agronomical evaluation program conducted with *P. maximum* in Brazil demonstrated that several important traits deserve genetic improvement. Many high-yielding forage accessions can be directly selected from the introduced germplasm, and some will be released in the next five years. Very few, if any, combine high herbage production with traits like:

- determinate flowering habit,
- nonshattering seeds,
- good quality seeds,
- insect resistance,
- adaptability to low fertility savanna soils,
- drought tolerance,
- rapid seedling growth,
- creeping habit with rooting at the nodes,
- forage quality, high palatability and digestibility.

Nature and inheritance of apomixis

Apomixis in *Panicum maximum* has been characterized as the result of apospory, i.e. embryo sac development from a somatic cell, followed by parthenogenesis, i.e. development of an embryo from the unfertilized egg cell (Warmke, 1954; Combes, 1975; Savidan, 1982a).

Breeding of any apomictic species needs availability of totally or highly sexual plants. Sexuality in *P. maximum* was first discovered by Combes & Pernes (1970) in diploid accessions from East Africa. Smith (1972) and Hanna et al. (1973) selected highly sexual tetraploids from South African apomictic accessions. Combes and Pernes' sexual diploids were treated by colchicine, and a few tetraploid plants were obtained, which showed to be entirely sexual.

Sexual and apomictic plants can be screened at two different levels. Progenies from apomicts are homogeneous and show the maternal phenotype. Sexual ecotypes are crosspollinating and produce highly heterogeneous progenies. A far more rapid determination of the reproductive behaviour can be obtained from gametogenesis analyses on the mother plant itself. Apomictic plants usually show multiple embryo sacs in a single ovule, as several nucellar cells differentiate at the same time. Above all, aposporic embryosacs have a 4-nucleate structure: one egg, two synergids and a single polar nucleus. Sexual plants never show more than one embryo sac per ovule, which is always of the 8-nucleate *Polygonum* type.

Using sexual tetraploids as female, and apomictic accessions as pollinators, a genetic analysis of apomixis and sexuality in *Panicum* was started in 1971. The main results are summarized in Table 1. From these data, a single dominant gene hypothesis has been presented (Savidan, 1982a; 1983) in which gene A controlled apomixis, and Aaaa was the genotype of all the tetraploid apomicts tested in crossing experiments. Sexual genotypes were aaaa. Sexual × apomict crosses made in Japan, using sexual tetraploids which also originated from natural diploids, gave segregations in agreement with this model (Nakajima & Mochizuki, 1981). Similar hypotheses of a simple genetic control for apomixis have already been discussed for the *Bothriochloa-Dichanthium* complex (Harlan et al., 1964), *Cenchrus ciliaris* (Taliaferro & Bashaw, 1966; Read & Bashaw, 1969), and *Eragrostis curvula* (Voigt & Bashaw, 1972). More recently, preliminary data from interspecific hybridizations in *Brachiaria* (Ndikumana, 1985), suggested a similar simple inheritance. Be that as it may, all these data strongly demonstrated an easy manipulation of modes of reproduction in tropical forage grasses. Breeding of such apomictic species is therefore no utopia.

Breeding procedures

Genetic improvement of apomicts can be obtained from crossing (1) sexual × apomict, when sexuality is available, (2) facultative apomict × apomict or (3) obligate apomict × apomict, when sexuality has not yet been discovered.

Breeding procedures for *Panicum maximum*, based on (1) sexual × apomict crosses, have been previously proposed by Smith (1975). These were based on limited knowledge of both natural variability and biology of *Panicum*, thus proving to be somewhat inappropriate for the species. Each hybrid generation required a 3 year period: the first year for making the crosses, the second one for growing the hybrids, and the third for progeny testing and screening of the apomicts. In Brazil we can accomplish hybridizations in May, plant the hybrids as soon as June, after removing the glumes and thus eliminating most seed dormancy, and finally screen embryologically for modes of reproduction by the first flowering. Therefore the whole process can be completed in no more than one single year. The proposed hybridization technique is simpler and far more productive than Smith's mutual pollination (Smith, 1975). We can make use of seed multiplication plots, by planting selected sexual progenitors in the middle. The sexual progenitors will be entirely sexual and never yield apomicts by selfing (Table 1), while the sexual plants used by Smith (1975) were highly facultative apomicts, with a mean production of only 55 per cent of hybrids in the progenies. Sexual progenitors must also be selected for high self-incompatibility. In all crosses made by Savidan (1982a) for the genetical analysis already mentioned, rates of selfing never overtopped 5 per cent. Techniques similar to Smith's mutual pollination were used in a 8 sexual × 8 apomict diallel cross (Savidan, 1980;

Chaume, 1985), resulting in higher rates of selfing, since crosspollination was less favored than in the plot technique. Selfed plants were easily separated from hybrids, due to inbreeding effects such as wrinkled leaves and general low vigor. In the plot technique, hybrid seeds were collected by shaking the sexual plant inflorescences above a paper bag three to four times a week. Mature seeds fall, which were later dried and stored at room temperature.

Transfer of any trait to a highyielding variety can follow selection schemes similar to those presented in Fig. 1. Several sexual plants are already under agronomical evaluation for selection of the best progenitors. Some seem good forage producers. Since most variability is presently encountered in apomicts, the A scheme (Fig. 1) is more likely to be used that the B scheme, at least until the sexual gene pool has been increased by the production of new sexual hybrids in F1 and further backcrosses. The breeding procedures discussed by Pernes et al. (1975) and presented in a simplified way in Fig. 2, intended to create some kind of ideotype by combining traits from several sexual and apomictic progenitors. It has been applied with some success in Africa, increasing variation in available sexual genotypes and producing some good new apomictic germplasm. Obviously it does not apply to the Brazilian situation, in which high yielding varieties are being selected in a first step of agronomical evaluation, which will need to be improved in a second step for one or a few definite traits.

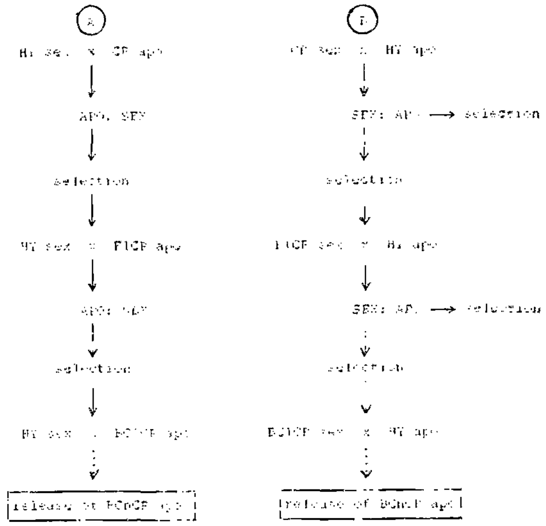

Fig. 1. Breeding schemes to transfer creeping habit (CR) to any highyielding (HY) ecotype. The A scheme starts with sexual HY, while the B scheme starts with apomictic HY and appears to be more time consuming since both sexual and apomictic hybrids needs to be selected at each generation.

Improvement through (2) facultative apomict × apomict crosses, or (3) obligate apomict × apomict crosses, are only means to escape from despair when true sexual plants are not available. Facultative apomicts usually produce low rates of off-

Table 1. Segregations observed between apomictic and sexual hybrids in ten different types of progenies. There is no significant difference (P<.05) with ratios expected from the single dominant gene hypothesis. From Savidan (1983)

Cross combinations	No. plant	Observed		Theoretical		
		apo	sex	apo	sex	
F1 = S1 × A1	133	71	62	67	66	NS
3W* = F1S × A2	279	135	144	139	140	NS
F1S selfed	126	0	126	0	126	NS
3WS selfed	57	0	57	0	57	NS
BC* = F1S × A1	26	14	12	13	13	NS
BC* = 3WS × A2	170	73	97	85	85	NS
FS = 2WS × 3WS	82	0	82	0	82	NS
FS* = 3WS × 3WA	60	26	34	30	30	NS
TC* = S1 × F1A	23	13	10	12	11	NS
3WA × 3WA	71	53	18	53	18	NS

* 3W: 3-way hybrids, BC: backcrosses, FS: full-sib, TC: test cross. For the 3WA × 3WA crosses, a facultative apomict was used as female, and only off-types were analyzed.

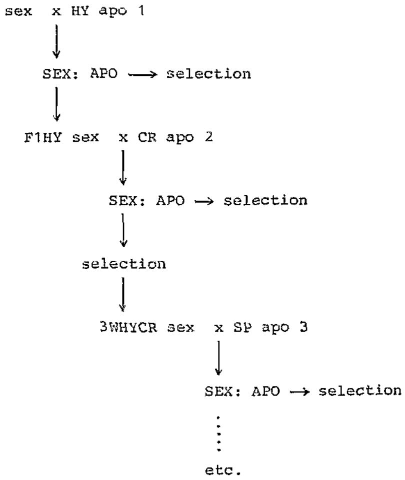

Fig. 2. Breeding scheme to combine several traits such as high forage production (HY) to creeping habit (CR) and good quality seed production (SP). Adapted from Pernes et al. (1975).

types (Savidan, 1982b), resulting from fertilization of either reduced meiotic or unreduced aposporic egg cells. Even when failure of meiosis is complete, one can expect some progress from the selection of accessions with a high rate of (2n + n) genotype production. Breeding through obligate apomict × apomict crosses may seem an extravagant proposal, but one must remember that even in obligate apomicts the endosperm is produced after fertilization of the unreduced polar nucleus. This means that hybrid endosperms can be produced, therefore allowing improvement for seed weight, seed dormancy, and possibly rapid seedling growth.

Conclusions

The *Panicum maximum* germplasm should be considered as a first opportunity for Brazilian plant geneticists to breed an apomictic species. Classical recipes for crosspollinating or selfpollinating species do not apply. New schemes need to be tested.

Apomixis has long been merely regarded as an impediment to plant breeding. Comprehensive studies on apomictic plant populations, biology and reproduction, showed that far from being the dead-end presented by Darlington (1939), apomixis may be easily manipulated. Some of the advantages brought by apomixis, such as hybrid vigor fixation, simplification and consequent low cost in hybrid seed production, aroused the interest of many crop breeders in recent years. Transfer of apomixis to wheat, rice, maize, sorghum and millets, is currently being attempted. A first International Conference on 'The Potential Use of Apomixis in Crop Improvement' has been organized in 1986 by the Rockefeller Foundation, and apomixis research specialists gained their own newsletter, the 'Apomixis Newsletter'.* Apomixis is now rising as a new tool in plant breeding (Savidan, 1986). It is a characteristic of the Panicoideae subfamily of the Gramineae (Brown & Emery, 1958), therefore a characteristic of most tropical forage grasses. The need, for tropical countries, to develop and test breeding programs on apomictic forage species as a mean to increase beef and dairy cattle productions, justifies our present efforts to select apomictic and sexual progenitors of *P. maximum* for further crossing experiments.

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