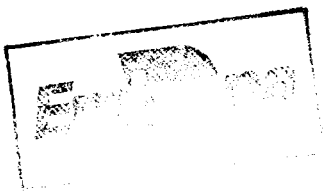

APOMIXIS AND SEXUALITY IN *BRACHIARIA DECUMBENS* STAPF

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INTRODUCTION

Brachiaria decumbens Stapf is a perennial tropical forage grass, native to east African savannas. It was introduced to Brazil in the 1960's and is now widely cultivated over 30 million hectares in Central Brazil alone. The two commercial varieties are apomictic tetraploids (2n = 4x = 36), producing uniform progeny, and were selected for their high dry matter production and persistence on poor acid soils. They lack, however, resistance to spittlebugs (*Deois* spp., *Zulia* spp., *Aeneolania* spp.) and produce forage of low nutritive value, especially during the dry winter months. The absence of natural variability associated to apomictic reproduction has prevented improvement of these varieties through hybridization. The *Brachiaria* germplasm recently collected by CIAT (International Center for Tropical Agriculture)/ILCA (International Livestock Center in Africa) under the auspices of IBPGR (International Board for Plant Genetic Resources) opens up new possibilities of selection of superior accessions and intra and interspecific breeding within this genus. The germplasm includes about 800 accessions of 24 different species of *Brachiaria,* collected over six countries in East Africa (Centro International de Agricultura Tropical, 1986). Breeding within this genus has been complicated by differences in ploidy levels between sexual and apomictic species (Ferguson, 1974), and by inherent difficulties in marking interspecific hybrids (Ndikumana, 1985). The study of the mode of reproduction of this *B. decumbens* germplasm was undertaken in hopes of finding sexuality and thus implementing an intraspecific breeding program aimed at producing superior apomictic cultivars to be released in Brazil.

MATERIALS AND METHODS

The 28 accessions studies are identified on Table 1. The mode of reproduction was determined by the study of the internal structure of the ovaries in young florets with recently extruded stigmas. These were fixed in FAA (Formaldehyde : Acetic Acid : Ethanol : Water in the proportions 3 : 3 : 40 : 14 v/v) for 24 hours at room temperature, then transferred to 70 % ethanol and refrigerated. The individual ovaries were dissected using a stereoscope, following dehydration and clearing using methyl salicytate (Young *et al.,* 1979). Pistils were then mounted in methyl salicylate, on microscope slides with unsealed cover slips and examined using interference contrast microscopy. The accessions were classified as sexual or apomictic according to the presence of meiotic (S), only, or aposporic (A) and multiple aposporic (Sm) embryosacs in their ovaries, respectively. A variable amount of unidentifiable sacs (?) were observed on both sexual and apomictic accessions and these included sterile, degenerated, ruptured, old or darkly stained pistils. A minimum of 25 ovaries per accession were examined. Chromosome counts were done on microsporocytes undergoing meiosis. For cytological analysis, young inflorescences were fixed in a freshly prepared solution of 3 : 1 absolute ethanol : propionic acid. Ferric chloride (1 g/100 ml of solution) was added to intensify the staining of chromosomes. Anthers were stained with 1 % propiono-carmin, warmed over an alcohol lamp and squashed for examination. Counts were taken on 25 to 30 cells per accession.

Table 1. Origin and identity of *B. decumbens* accessions.

SCPA Code*	CIAT Code	Origin	Country
BRA-004405	16489	Uasin Gishy	Kenya
BRA-004413	16490	Trans Nzoia	Kenya
BRA-004421	16491	Trans Nzoia	Kenya
BRA-004430	16493	Siaya	Kenya
BRA-004448	16494	Siaya	Kenya
BRA-004456	16495	Siaya	Kenya
BRA-004464	16496	South Nyanza	Kenya
BRA-004472	16497	South Nyanza	Kenya
BRA-004481	16498	Nandi	Kenya
BRA-004499	16499	Nakuru	Kenya
BRA-004502	16500	Uasin Gishy	Kenya
BRA-004511	16501	Trans Nzoia	Kenya
BRA-004529	16502	Trans Nzoia	Kenya
BRA-004337	16503	Trans Nzoia	Kenya
BRA-004545	16504	Nandi	Kenya
-	16519	Kwale	Kenya
BRA-004561	26182	Rutana	Burundi
BRA-004596	26287	Kigali	Rwanda
BRA-004667	26297	Kibongo	Rwanda
BRA-004707	26300	Byumba	Rwanda
BRA-004723	26302	Byumba	Rwanda
BRA-004731	26303	Byumba	Rwanda
BRA-004758	26305	Kigali	Rwanda
BRA-004782	26308	Butare	Rwanda
-	26568	África	-
-	26569	África	-
BRA-001058	606	cv. Basilisk (commercial)	
BRA-000191	6012	cv. IPEAN (commercial)	

*SCPA = Cooperative System in Agricultural Research (Brazil)

RESULTS

Study of megasporocytes revealed the formation of obligatory meiotic embryo-sacs of the *Polygonum* type on nine accessions. The eggcell or its very distinct and dense cytoplasm,

two polar nuclei and multiple antipodal cells were clearly visible and for these accessions aposporic sacs, which for *Brachiaria* are of the *Panicum* type (formed by one egg-cell, one polar nucleus and two short-lived synergids), were never observed (Table 2). These nine accessions are therefore, completely sexual in contrast to the remaining material that exhibited varied levels of sexuality. Some of the accessions showed sexuality as high as 38 % at the embryological level however levels lower than these are expected in field trials. The sexual accessions are diploids (2n = 2x = 18) showing normal bivalent association while the apomictics are tetraploids with predominant bivalent association and less frequent univalent, quadrivalent and trivalent associations in meiotic diakinesis and metaphase of microsporocytes.

Table 2. Embryo-sac analysis of *B. decumbens* accessions.

Accession*	Total ovaries	Percentage		Sacs**		Mode of reproduction
		S	A	Sm	?	
16493	30	60	0	0	40	Sexual
16494	28	89	0	0	11	Sexual
16495	30	80	0	0	20	Sexual
16519	28	86	0	0	14	Sexual
26302	27	89	0	0	11	Sexual
16303	25	88	0	0	12	Sexual
26305	32	75	0	0	25	Sexual
26308	30	73	0	0	27	Sexual
26287	33	6	0	0	94	Sexual?
606	42	22	33	38	7	Apomictic
6012	31	16	52	16	16	Apomictic
Mean of remainder	454	0-38	13-60	23-75	0-36	Apomictic

* CIAT code numbers

**S = meiotic — Sm = multiple aposporic

A = aposporic — ? = unidentifiable

DISCUSSION

The discovery of completely sexual accession (4 from Kenya and 4-5 from Rwanda) within a species considered as obligate apomitic allows for intraspecific breeding such as those accomplished in *Panicum* (Savidan, 1983 ; Hanna *et al.* ; 1973), *Cenchrus* (Taliaferro and Bashaw, 1966) and offers an alternative to the previously suggested methodology involving more tedious and less successful interspecific crosses in *Brachiaria* (Gobbe *et al.*, 1983 ; Ndikumana, 1985). The natural variability present in the germplasm at Campo Grande fulfills that prime requisite for a breeding program. The field nursery has been established and simultaneous to the cytogenetical and embryological examination, agronomic and morphological data to be used in numerical taxonomy, are being gathered following a scheme discussed elsewhere (Valle *et al.*, 1986). The sexual accessions in this collection will be used to introduce desired characteristics into the already well adapted cultivars (Figure 1). This program involves several stages and the goal is to obtain apomictic hybrids with agronomic potential as well as sexual hybrids to be crossed to superior apomictics. Agronomic and animal evaluation trials should follow, both within the research station and in regional trials in selected regions of Brazil before the apomictic hybrid is released.

CONCLUSIONS

Obligate sexuality was found for *B. decumbens* in the germplasm collected by CIAT/ILCA and introduced to Campo Grande, Brasil.

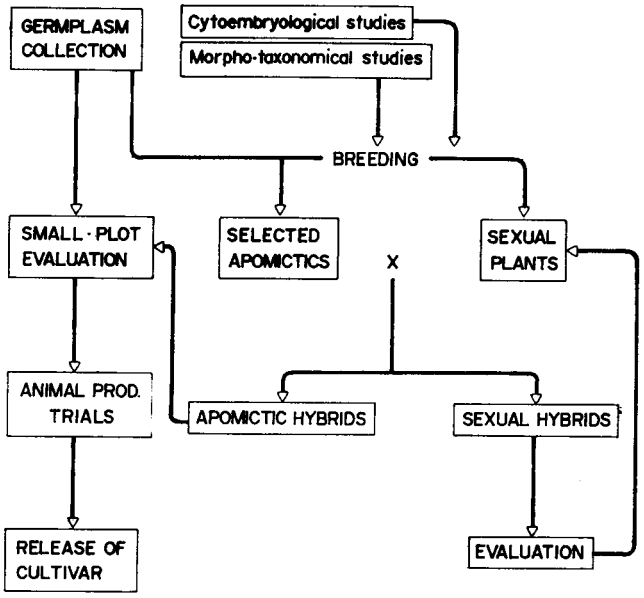

Figure 1. Breeding and evaluation scheme for *Brachiaria* in Campo Grande, Brazil.

The sexual accessions are diploids (2n = 2x = 18), while the recently introduced as well as the adapted commercial accessions are apomictic tetraploids. Hybridization among them will take place as soon as artificially induced tetraploids are obtained.

Presence of sexuality in *B. decumbens* should simplify considerably the breeding of this species, allowing for the development of superior apomictic hybrids.

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