# Cytology and reproductive behavior of diploid, tetraploid and hexaploid germplasm accessions of a wild forage grass: *Paspalum compressifolium*

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#### Summary

Fourteen germplasm accessions of *Paspalum compressifolium* native from southern Brazil were cytologically and embryologically analysed. The study revealed that one accession was diploid (2n = 20), twelve were tetraploid (2n = 40) and one was hexaploid (2n = 60). This is the first report of diploid and hexaploid cytotypes for this species. Studies on microsporogenesis, megasporogenesis, and embryo sac development indicated that the diploid cytotype had regular meiotic behavior and reproduces sexually. Tetraploid cytotype usually had an important proportion of chromosomes that associated as quadrivalents during meiosis and reproduced by mean of aposporous apomixis. The hexaploid cytotype showed irregular meiotic behavior with about one third of the chromosomes associated as multivalents and reproduced by aposporous apomixis. Thus, *P. compressifolium* could be an agamic complex. Breeding in this complex is possible due to the presence of diploid sexuals which can be treated by colchicine and pollinated by apomicts.

#### Introduction

Paspalum compressifolium Swallen is a South American grass belonging to the Plicatula group of Paspalum (Chase, 1929; Swallen, 1967). Most species of this group are important forage constituents of the native grasslands in South America, especially in southern Brazil, northeastern Argentina and eastern Paraguay.

Honfi et al. (1990) reported that *Paspalum compres*sifolium has 40 chromosomes. Nevertheless, different chromosome races is a common feature in species of this genus. Usually, diploids reproduce sexually, while tetraploid counterparts are apomictic (Quarín, 1992). Agamic complexes with sexual diploids and apomictic polyploids are generally concordant with taxonomic groups of the genus. Diploids are of special interest as sources of sexuality in our germplasm collections of *Paspalum*, a genus with predominance of apomictic polyploid biotypes.

Our paper shows how *P. compressifolium* has the main traits of an agamic complex. Results concern: i)

the chromosome number and ploidy level, ii) the meiotic chromosome behavior, and iii) the reproductive system.

### Material and methods

The fourteen studied accessions were collected in different collection trips throughout the states of Paraná, Santa Catarina, and Rio Grande do Sul, Brazil, embracing the distribution area of *P. compressifolium*. Table 1 shows the collection locality, the Brazilian plant germplasm number, and the herbarium number for each accession. All accessions are held in the gene bank of CENARGEN at Brasilia, Brazil. Herbarium vouchers of Valls' collections are deposited at CEN and those of Krapovickas at CTES.

Plants were grown in pots in glasshouses. Methods used to study mitosis, meiosis, embryo sac development, and determination of seed set were similar to those reported by Quarín & Hanna (1980). Pollen ger-

Table 1. Collection site and chromosome number of fourteen Paspalum compressifolium accessions from Brazil

Accession <sup>1</sup>	BRA <sup>2</sup>	Origin <sup>3</sup>	2n
VBoPrSe 4225	008516	Arroio dos Ratos, RS	20
VNSh 4084	008508	Vacaria, RS	40
VCoBu 4400	008524	Vacaria, RS	40
VCoBu 4495	008532	Lages, SC	40
VDGoMi 8070	005088	Urubici, SC	40
VDGoBD 8098	012238	Bom Retiro, SC	40
VGoMi 10733	012282	Passo Fundo, RS	40
VGoMi 10744	011355	Lagoa Vermelha, RS	40
VGoMiSv 11100	013102	Balsa Nova, PR	40
VGoSv 11179	013455	Balsa Nova, PR	40
VGoSv 11449	014371	Palmas, PR	40
K 40758		Ponta Grossa, PR	40 <sup>4</sup>
K 40811	-	Vila Velha, PR	40 <sup>4</sup>
VGoMiSv 11081	013030	Campo Largo, PR	60

<sup>1</sup> Collectors: Bd = I.I. Boldrini; Bo = S.C. Boechat; Bu = B.L.Burson; Co = L. Coradin; D = M. Dall'Agnol; Go = K.E. Gomes; K = A. Krapovickas; Mi = S.T.S. Miotto; N = C. Nabinger; Pr =A.I. Pereira Flores; Se = B.A. Severo; Sh = V.P. Schell da Silva, Sv = G.P. da Silva; V = J.F.M. Valls.

<sup>2</sup> Brazilian plant germoplasm number.

<sup>3</sup> PR, RS, and SC mean the Brazilian States: Parana, Rio Grande do Sul, and Santa Catarina respectively.

<sup>4</sup> Reported by Honfi et al. 1990.

mination and pollen tube growth following self- and open-pollination were surveyed according to the technique proposed by Kho & Baër (1968).

## Results

#### Cytology and embryology

Twelve accessions were tetraploid (2n = 4x = 40), one was diploid (2n = 2x = 20), and one hexaploid (2n = 6x = 60) (Table 1). Meiosis of the diploid accession was regular showing 10 bivalents at diakinesis and metaphase I (Table 2 and Figure 1A), except three cells with 2 univalents and 9 bivalents. Three tetraploid accessions, sampled for meiosis, had irregular behavior with univalent, bivalent, and multivalent chromosome configurations (Table 2 and Figure 1B). The hexaploid accession showed a meiotic behavior with a range of chromosome configurations from univalents to hexavalents (Table 2 and Figure 1C). All these results emphasize clearly that polyploid types showed all meiotic features of autopolyploids, and that *P. compressifolium* 



Figure 1. Meiotic pairing configurations in Paspalum compressifolium at three ploidy levels. A, diploid cytotype (VBoPrSe 4245), metaphase I with 10 II. B, tetraploid cytotype (K 40758), diakinesis with 3 I + 3 II + 1 III (arrow) + 7 IV. C, hexaploid cytotype (VGoMiSv 11081), late diakinesis showing 21 II + 3 IV (arrows) + 1 VI (arrowhead).  $\times$  1500.

is a species complex characterized by different ploidy levels.

Megasporogenesis and embryo sac development were normal in the diploid accession. Meiosis produced a linear tetrad of megaspores. The three megaspores nearest the micropyle degenerated. The chalazal

Accession	2n	No.	Average/PMC (Range in parenthesis)						
		PMCs	I	II	III	IV	V	VI	
VBoPrSe 4245	20	81	0.07	9.96	-	-	-	-	
			(0–2)	(9–10)	-		-	-	
VDGoMi 8070	40	19	1.9	17.7	0.2	0.4	-	0.1	
			(0–4)	(15–20)	(0-1)	(02)	-	(0-1)	
K 40758	40	12	1.8	8.8	0.4	4.8	-		
			(04)	(3–15)	(0–2)	(1-8)	-	-	
K 40811	40	26	0.7	9.8	0.2	4.8	-	-	
			(0–3)	(4–15)	(02)	(27)	-		
VGoMiSv 11081	60	30	2.4	19.5	1.0	2.7	0.1	0.7	
			(09)	(9–28)	(0-4)	(06)	(0-1)	(0–2)	

Table 2. Meiotic chromosome configurations of different cytotypes of Paspalum compressifolium at diakinesis and metaphase I

cell, which was functional megaspore, developed into an embryo sac characterized at maturity by: the egg apparatus (egg cell and two synergids); a large widely vacuolate central cell with two nuclei, both laying at the end near the egg apparatus; and a mass of antipodal cells characteristic of most sexually reproducing grasses.

Megasporogenesis and embryo sac development in hexaploid and tetraploid accessions differed from diploids. The archesporial cell developed from a hypodermal cell of the nucellus in the ovule and underwent meiosis to produce a linear tetrad of megaspores. All four megaspores degenerated while one to seven nucellar cells began enlarging to form at maturity one to two fully developed aposporous embryo sacs in each ovule. These sacs had generally one egg cell, two syndergids, and a large globular 2-nucleate cell. Usually, several other small aposporous embryo sacs bearing one to four nuclei surrounded the one or two well developed sacs. Proembyos were observed in mature sacs of several ovules (15%) collected at anthesis, but only in the hexaploid accession. Nevertheless, no endosperm development was observed in these sacs, previous to anthesis.

Data from cytological and embryological studies suggest that *Paspalum compressifolium* has several traits of an agamic species with the aposporic type of apomixis. Clearly there are two pools, a diploid sexual one and a polyploid apomictic one.

# Phenology, compatibility and fertility

All three cytotypes had similar heading period at Corrientes: from the end of December to middle March. Usually a group of spikelets of each inflorescence (head) bloomed in every twenty-four-hour period. Commonly four to five days elapsed between the initiation of anthesis and the last flowering spikelets of the head. Anthesis occurred daily from 7:00 to 9:00 AM in the hexaploid and from 2:00 to 5:00 PM in diploid and tetraploid cytotypes. Each spikelet opened, the anthers and stigmas came outside, and the anthers dehisced, all in approximately 15 minutes. The spikelets closed soon after the pollen was shed.

Seed set was investigated on diploid (4245), tetraploid (40758 and 40811), and hexaploid (11081) plants cultivated at Corrientes, Argentina. Only one clone for each accession was cultivated and all of them grew close together in the field. No seed formation was observed in the diploid clone suggesting a selfincompatibility system. Tetraploids and the hexaploid produced seed at low rates (10 to 25%) under both conditions: self and open pollination showing absence of self-incompatibility.

Studies through fluorescence technique showed that pollen grains germinated and pollen tubes penetrated the stigma papillae few minutes after landing in all three cytotypes following self pollination. However, in the diploid plant, the pollen tubes failed to penetrate the style showing the presence of a selfincompatibility system. In polyploids, several pollen tubes developed through the stigma, the style, and the ovary. They penetrated the ovule through the micropyle about three hours after loading of self pollen on the stigma. In tetraploids, pollen grain germination and pollen tube growth showed similar behavior after crosspollination. Presence on only one diploid and only one hexaploid did not allow cross-pollination. Hand emasculated inflorescences of polyploid plants failed to set seed indicating the incidence of pseudogamy in seed formation.

Paspalum compressifolium is concerned with selfincompatibility in the sexual diploid pool and with selfcompatibility in the pseudogamous aposporic polyploid pool.

# Discussion

Presence of several ploidy levels, autopoliploidy, and aposporic embryo sac in P. compressifolium are typical traits of aposporic complexes. Indeed, P. compressifolium is a part of a major agamic complex that embraces the Plicatula group of Paspalum. Our study confirms the strong relation between gametophytic apomixis and polyploidy and their incidence in the evolution of important tropical and subtropical grass genera. A well known example concerns Dichanthium, Bothriochloa, and Capillipedium, three closely related genera of the tribe Andropogoneae, which integrate an agamic complex joined by the campilospecies B. intermedia. According de Wet and Harlan (1970) this complex includes rare sexual diploids and facultative apomictic polyploids. Bothriochloa is further characterized by sexually reproducing polyploids. A similar genetic system with diploid-tetraploid-dihaploid cycles was also reported for Panicum maximum, an agamic complex of the grass tribe Paniceae (Savidan & Pernès, 1982).

Our results suggest a similar pattern for the genetic system of *Paspalum compressifolium* and the agamic complex to which it belongs: the Plicatula group of *Paspalum*. Similar to *Dichanthium* and related genera, we also found two pools: one sexual, diploid and selfsterile, and the other pseudogamous apomictic, polyploid, and self-fertile. These pools are disymetric with rare sexuals and frequent apomictic polyploids. The morphological similarities among diploid a tetraploid cytotypes of *P. compressifolium* as well as the frequency of multivalent associations at meiosis in the tetraploids strongly suggest an autotetraploid origin, and confirms the close relation between apomixis and autoploidy in the genus *Paspalum* as pointed out previously by Norrmann et al. (1989).

The hexaploids in *P. compressifolium* are also rare and the existence of some differences in morphology and flowering behavior in relation to diploid and tetraploid cytotypes suggest interspecific hybridization in their origin. Fertilization of cytologically unreduced gametes may favor hybridization of two apomictic tetraploid species within the agamic complex to produce an apomictic hexaploid.

Gustafsson (1947) pointed out that gametophytic apomixis is strongly associated either with dioecismmonoecism or self-incompatibility. Apomixis occurs more readily in cross-fertilizing than in self-fertilizing plants. Unfortunately, as properly indicated by Asker and Jerling (1992), too little is known concerning the occurrence of incompatibility mechanisms in sexual relatives of agamic complexes. Nevertheless, recent studies in Paspalum showed that most apomicts are autotetraploid, whereas the related sexuals are diploid and self-incompatible (Norrmann et al., 1989; Quarín, 1992). The present study confirm this pattern for one more species of Paspalum and allow to predict that wherever a diploid self-incompatible species exists, an apomictic autotetraploid co-specific counterpart could likely be found. In the particular case of P. compressifolium, an hexaploid was occasionally formed, probably through a B<sub>III</sub> interspecific hybridization process.

Since polyploid cytotypes are apomicts, little improvement can be expected from a standard breeding program. However, tetraploids offer the opportunity to be used as male parents if we are able to double the chromosome number of the diploid plant producing a sexual tetraploid to be used as female as in other apomictic breeding programs (*Panicum, Brachiaria*).

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