Cytogenetical and Reproductive Studies in the South American Grass *Echinochloa helodes* (Hackel) Parodi

V. Ferreira, B. Szpiniak and L. Reynoso

Genetics, Dept. of Agricultural Biology, Fac. of Agronomy and Veterinary,
University of Río Cuarto, 5800 Río Cuarto, Córdoba, Argentina

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The genus *Echinochloa* (*Panicaceae*, *Gramineae*) comprises ca. 40 species (Webster 1988), some of them have a varied geographic distribution throughout the world while others live in more restricted habitats (Yabuno 1975). Most of them are wild species, many grow in cultivated rice fields as serious weeds while others have a good grain or forage value. In many reports about biosystematics, Yabuno (1953, 1966, 1976, 1983a, b) pointed out that this genus is complex and polyploid, with basic chromosome number $x=9$, species from $2x$ to $14x$ and frequent presence of numerical cytotypes.

Echinochloa helodes (Hackel) Parodi (lagoon grass) is a native, perennial, rhizomatous, semihalophyte and hygrophylic Summer grass of temperate South America. It grows naturally in uncultivated, wet and swampy fields and it is palatable to cattle. Its distribution is restricted to the area between 26° – 35° S and 52° – 64° W approximately, in Uruguay, Northern and Center East of Argentina and Southern part of Brazil (Martínez Crovetto 1942, Burkart 1969, Rosengurtt *et al.* 1970, Cavalheiro 1977). The species has both reproductive systems: sexual and asexual. During seasonal growth, individuals develop fertile panicles and new starchy rhizomes from rooting stolons. Sexual seeds are easily scattered and limits the use of this species as a cultivated forage grass.

Thorough botanical descriptions of the taxon are available (Martínez Crovetto 1942, Burkart 1969, Cavalheiro 1977, Cantero *et al.* 1991), but data about its cytogenetics and sexual reproduction are scarce. On the basis of our previous observations (Ferreira *et al.* 1992) that a population in the Province of Córdoba (Argentina) showed 27 II in diakinesis and evidence of allogamy, we analyze in this paper chromosomic variants, sexual reproduction and seed retention in four collections of Argentina and Uruguay.

Materials and methods

Four populations collected in Santa Fe, Córdoba and Corrientes provinces (Argentina) and Rocha department (Uruguay) were used. Young panicles of 5 individuals per location were fixed in 6:3:1 (alcohol-chloroform-acetic acid) during 24 hrs and were stored in 70° alcohol at 4° C. Meiosis of 20–25 pollen mother cells (PMC) and 40–50 tetrads per plant were observed, using anthers processed by the acetocarmine squash method. The same coloring was used to determine fresh pollen stainability in 15–20 plants per population, two panicles per plant and 200 grains per panicle.

In order to study sexual reproduction, 8 plants were isolated in the field to avoid pollen flow. Panicles of 5–10 plants per population were isolated by bagging before blooming and 15–30 were allowed to pollinate freely and then bagged to avoid seed fall; besides, 10 panicles pairs

were bagged together and shaken once a day during a week. Spikelet number and seed set per panicle were counted and the percent of fertile flowers calculated.

All individuals available in the experimental field and two natural populations were observed to identify seed retention. Besides, mutagenic treatments were applied: 300 stolons were put to root in a solution of sodic azid (1×10^{-2} M), 500 stolons were irradiated with 1000 and 2000 rads of X-rays for 5 and 10 min and 350 seeds with 2000 rads for 20 min. All of them were planted or sown in a glasshouse. The first panicles of the 20 plants that bloomed that first year were fixed and analyzed with the above-mentioned cytology method. Panicles were jointly bagged in order to obtain sexual progenies.

Results

Meiotic divisions of the 410 PMC proceeded normally; 27 bivalents were observed in diakinesis or MI (Fig. 1a-c), mostly ring-shaped and a few rod-shaped. Migration at early AI was 27:27 without bridges or fragments. No micronucleoli were found in 920 tetrads and free microspores had up to two nucleoli. The average of pollen stainability was 93.4% (Table 1) with a range from 78.8 to 98.4%.

The flower structure confirmed protogyny. Fertile spikelets in isolated plants were 1.6% but the seeds did not germinate. The panicles that were isolated by bagging showed turgid stigmas for around ten days (Fig. 2), as a whole, in the four populations, 10.5% of fertile spikelets were obtained with large differences between them (Table 1). A hundred seeds were tested for germination using soil and inorganic material; only two germinated and grew to adult stages.

Open pollination as a whole (Table 1) yielded 53.5% of fertile spikelets, with large ranges of variation within populations, plants and panicles of the same plant. A good set of plants was obtained although germination tests did not exceed 30%. When panicles of different plants of a population were enclosed in the same bag, seed set decreased to 25.7%.

The rachillas of all individuals observed

Fig. 1. Meiotic pairing in *Echinochloa helodes*: a) diakinesis showing 27 II; b) diakinesis, two pairs are associated to the nucleolus; c) MI, 27 II. The scale represent 2 μ .

Table 1. Pollen stainability and fertility in *Echinochloa helodes* (Hackel) Parodi

PO	Stainable pollen		a	b	Self pollination					a	b	Cross pollination				
					c	d			c			d				
	%	Range				N°	%	Range				N°	%	Range		
SF	94.5	(82.1–97.7)	8	14	590	61	10.3	(0.0–18.2)	20	38	1716	992	57.8	(25.0–80.1)		
CO	93.2	(78.8–98.4)	12	22	898	104	11.6	(0.0–25.0)	29	103	4864	2335	48.0	(9.7–74.3)		
CS	94.1	(84.2–98.1)	5	10	390	40	10.2	(0.5–16.2)	15	25	1120	624	55.7	(19.2–76.5)		
RO	91.8	(80.6–95.2)	8	12	452	44	9.7	(0.0–16.1)	18	20	810	426	52.6	(11.4–72.6)		
x/T	93.4	(78.8–98.4)	33	58	2330	249	10.5	(0.0–25.0)	82	186	8480	4377	53.5	(9.7–80.1)		

PO, Population: SF, Santa Fe, CO, Córdoba; CS, Corrientes (all Argentina); RO, Rocha (Uruguay), x/T, mean or total: a=plants; b=panicles; c=spikelets; d=seeds.

Fig. 2. Plants of *Echinochloa helodes* with open and self-pollinated panicles. Height 70–80 cm.

in the experimental and natural fields shattered quickly and did not retain seed (Fig. 3a–d). The stolons treated with mutagenic agents showed high mortality and only two seeds germinated but their seedlings died. Only 143 plants were obtained from the irradiated stolons, they had not any morphological variants, 24 plants flowered at the end of Summer and no seed were retained.

Twenty of them showed normal meiosis. The progenies had normal morphology.

Fig. 3. Panicles of *Echinochloa helodes*. a) blooming stage; b) shattering after 10 days; c) rachis after two weeks; d) rachis nude. Scale $\times 1/4$.

Discussion

Six species of the genus *Echinochloa* live naturally in Argentina and Uruguay. Three are American taxa: *E. crusgavonis* (H.B.K.) Schultes (annual), *E. polystachya* (H.B.K.) Hitchcock (perennial) y *E. helodes* (Hackel) Parodi (perennial). The first two are widely distributed from the South of USA down through Central Argentina; the latter grows in a more restricted area: Uruguay, Northern and Center East of Argentina and South of Brazil. Its natural habitats are lowlands and depressed plains with sometimes salty soil. It is a weed in rice fields but has good

forage value (Martínez Crovetto 1942, Burkart 1969, Rosengurtt *et al.* 1970).

This genus is a polyploid series with $x=9$ (Yabuno 1953, 1966, 1983b) and *E. helodes* is $2n=6x=54$ (Ferreira *et al.* 1992). The four collections here used have the same chromosomal number. All analyzed plants showed meiotic normality. In fact, at our present state of knowledge, this species does not have numerical cytotypes in contrast with other perennial species of the genus, as *E. stagnina*, *E. pyramidalis* and *E. haploclada* (Yabuno 1968, 1978, 1983a, b).

The origin of the hexaploid grass *E. helodes* is unknown. Meiotic behavior does not show any evidence of autopolyploid. This situation could only occur if the taxon had a strong genetic mechanism to avoid multivalent pairing. On the other hand, there are no heredity studies of Mendelian characters; therefore, segregating ratios to indicate autopolyploid are not available.

By contrast, studies by Yabuno (1953, 1968, 1976, 1978, 1983b) on interspecific crosses suggest that many species of the genus are allopolyploid. Meiotic regularity, normal tetrads and high stainable pollen suggests that *E. helodes* is an ancient allopolyploid with an advanced diploidization process. In addition, this taxon has a complete reproductive isolation, at least in front of *E. crus-galli*, annual and $2n=6x=54$, as shown by our negative results in hybridization attempts (unpublished results).

In diploid Angiosperms, the presence of only one nucleolus is the general rule, but its number in polyploids is normally higher. The maximum number of nucleoli observed during its formation revealed regions of RNAr synthesis (Anastassova-Kristeva *et al.* 1977) which is carried out by the SAT chromosomes in MI. *E. helodes* has a maximum of two nucleoli in free microspores, this fact suggests that the taxon has at least two pairs of chromosomes with NOR region.

Knowledge of sexual reproduction is essential in order to choose a breeding method. Only clonal selection has been available although breeding attempts have not been made. This paper describes the sexual reproductive system. Protogyny has been confirmed through botanical descriptions and the flower structure observations, which indicates that dychogamy is a sound reference to allogamy (Allard 1967). Failure of isolated plants in the field to produce seed strongly supports this statement.

The panicles isolated by bagging accumulated good quality pollen grains in the bag and the stigmas remained receptive for about ten days. Few flowers were fertilized, the seed set was poor (10.5%) and had very low viability. This fact confirms the presence of a low percent of self-fertility and is in agreement with high outbreeding.

The seed set for free pollination was 53.5% with a large range of variation and confirms allogamy prevalence. Under natural conditions, rachillas broke easily and the seeds of the first fertilized flowers began to fall before the last ones ripened. Then, it can be assumed that some deficiencies in the bagging technique to avoid seed fall could have affected the seed set. Moreover, this taxon has a long flowering time, from Summer to the beginning of Autumn, sometimes with pollen shortage. This may contribute to the fecundation failure in small stands (Levin 1988).

When two panicles of different plants were placed in the same bag, some of the seeds might have been produced by the scarce self-pollination, but most of them must have been derived from cross-pollination. Nevertheless, seed set was about half in contrast with free pollination and suggests the presence of partial self-incompatibility as some species of the genus show (Yabuno 1983a).

Mixed mating systems with different degrees of genetic and environment components is not surprising, in Nature there are many examples (Silvertown and Lovett Doust 1993). The effect of the environment on sexual reproduction of lagoon grass might be important. The habitat of the species has frequent flood-drought cycles, with water excess in Spring and Autumn and

occasional deficits in the warmer Summer months.

Under such conditions, a drought stress at blooming may temporarily reduce the effective number of reproductive individuals in a population. Consequently, natural selection could favor genic combinations to promote autogamy in early blooming individuals (Wyatt 1988). The same effect could also happen in the earliest or latest blooming plants without special conditions.

Lagoon grass is a wild species with a specific habitat. In some wet and swampy areas it is a good forage resource. Cattle prefer it to other forage plants, which accounts for the limited clonal growth dispersion, seed production and dispersal are strongly dependent on cattle management. The forage characteristics of lagoon grass make its improvement desirable. But sexual seeds to sow or to design and implement a breeding program are not available because they are not retained.

A few species of *Echinochloa* are used as a grain crop (Yabuno 1962). A Caucasian race of *E. oryzoides* known as *E. macrocarpa* and a French strain of the same species shows seed retention (Yabuno 1984). Thus, this character is present in the genus. In *E. helodes*, no seed retention was found in natural and treated material, the latter has not shown morphological changes either. The possible chromosome damage induced by the X-ray treatments may be used as a hint of their effectiveness. Meiotic analysis of treated plants showed normal chromosome pairing, therefore, the treatment was ineffective or the damage was quickly repaired.

The amount of analyzed material in search of mutations was low, but no evidence of its occurrence was obtained. The mutagenic treatments in somatic tissue of polyploids normally give a few mutations, if any. Some reasons explaining this failure are: the ploidy level, inadequate radiation dose, exposition time and development of the putative mutagenic tissue in sexual reproductive organs. Furthermore, the negative effects of the treatment causing damage on seed viability was added to natural low germination. More research needs to be done about the seed physiology of this species in order to obtain effective mutagenic treatments.

To conclude, there are no chromosomal variants in the analyzed populations of *E. helodes*, outcrossing is the prevalent form of sexual reproduction and no seed retention has been found up to the present.

Summary

Echinochloa helodes (Hackel) Parodi grows naturally in Argentina, Uruguay and Brazil. It is a perennial, semihalophyte, rhizomatous and hygrophylic Summer grass, in naturally wet lands it is a good forage resource. This paper analyzes numeric chromosomal variants, sexual reproduction and seed retention in four populations of Argentina and Uruguay, through the study of meiotic behavior, pollen stainability and seed production in self- and open-pollinated panicles. All collections showed 6x level, with 27 bivalents at diakinesis-metaphase I and normal migration 27:27 in early anaphase I. Pollen stainability was 93%. Seed set was poor (10.5%) in self-pollinated panicles, but over 53% after open pollination, indicating the prevalence of allogamy. No seed retention was found.

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