Plant Syst Evol
DOI 10.1007/s00606-015-1206-1

ORIGINAL ARTICLE

Reproductive biology of *Sporobolus phleoides* Hack. (Poaceae), an endemic halophyte grass of Argentina

Geraldina Alicia Richard[1,3] · **José Francisco Pensiero**[1,3] · **María Carolina Cerino**[1,3] · **Beatriz Gloria Galati**[2,3] · **Hugo Francisco Gutiérrez**[1]

Received: 13 June 2014 / Accepted: 9 March 2015

Abstract *Sporobolus phleoides* is an endemic grass of Argentina considered an important genetic resource for saline environments due to its salinity tolerance. Basic information about its reproductive biology is needed to adequately preserve its genetic variability. Hence, we studied its breeding system through phenology, embryological analysis, and self-pollination versus open-pollination treatments in six populations collected from four provinces of Argentina. The results indicated that *S. phleoides* is mainly autogamous with a high fertility rate. Cleistogamous and chasmogamous spikelets are isomorphic and occur simultaneously on the inflorescence, with higher percentages of cleistogamy found in the basal region of the panicle. The average percentage of cleistogamy during the study period was 23 % for all populations evaluated. The development of the embryo sac follows the typical pattern of grasses and indicates normal sexual reproduction. This study provides the basis for programming genotype collections for gene banks and future plans of domestication.

Handling editor: Jim Leebens-Mack.

✉ Geraldina Alicia Richard
geralrichard@gmail.com; grichard@santafe-conicet.gov.ar

[1] Cátedra de Botánica Sistemática Agronómica, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, RP Luis Kreder 2805, 3080HOF Esperanza, Argentina

[2] Cátedra de Botánica General, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina

[3] Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

Keywords Autogamy · Breeding system · Cleistogamy · *Sporobolus*

Introduction

Sporobolus R.Br. (Poaceae, Chloridoideae, Zoysieae) is a cosmopolitan genus of about 150 species that grow in tropical, subtropical, and warm-temperate regions throughout the world (Watson and Dallwitz 1992; Ortiz-Díaz and Culham 2000; Peterson et al. 2001). More than 70 species are distributed in America (Peterson et al. 2003, 2007), and 11 are represented in Argentina (Denham 2012). Many species of this genus are halophytes (Liphschitz and Waisel 1974), and most of them are used as forage resources, in re-vegetation plans, or as models for studies of salinity tolerance (Wood and Gaff 1989; Joshi et al. 2005; Khan and Gul 2008). Among these, it is found *Sporobolus phleoides* Hack., an endemic grass from north-central regions of Argentina, that integrates halophytic communities with species of recognised saline toleration of the genera *Distichlis* Raf. and *Sarcocornia* (Duval-Jouve ex Moss) A.J.Scott. This species is considered an important genetic resource and a potential fodder plant for saline environments (Aronsen 1989; Rogers et al. 2005). Knowledge of its reproductive system is required for the proper conservation, maintenance, and utilisation of its genetic diversity.

Studies on reproductive biology of the genus *Sporobolus* are scarce. Species are mainly sexual (Engstrom 2004), and some are capable of both sexual and vegetative reproduction (Smith-White 1988; Febles et al. 2010) due to the development of rhizomes and stolons (Smith-White 1988; Febles et al. 2010). Sub-sexual reproduction through agamospermy was also observed in a triploid race of *Sporobolus virginicus* (L.) Kunth (Smith-White 1988).

The presence of cleistogamy was recorded in many species of the genus, such as *Sporobolus platensis* Parodi (Rosengurtt and Arrillaga de Maffei 1961; Rosengurt 1984), *Sporobolus neglectus* Nash. (Doyon and Dore 1967), *Sporobolus asper* (P.Beauv.) Kunth (Riggins 1977), *Sporobolus indicus* (L.) R.Br. (Astegiano 1986), *Sporobolus ozarkanus* Fernald, *Sporobolus vaginiflorus* (Torr. ex A.Gray) Alph. Wood (Mc Gregor 1990), and *Sporobolus cryptandrus* A.Gray (Parodi 1928). This is a clear indicator of reproductive self-compatibility (Connor 1979, 1981); however, this was only checked in *Sporobolus airoides* (Torr.) Torr., *S. cryptandrus* (Fryxell 1957), *S. asper* (Weaver 1954), and *S. indicus* (Aracne 2010). These last two species are mainly self-pollinated; nevertheless, they have a mixed reproductive system in where self-pollination and cross-pollination coexist in the same panicle (Riggins 1977; Astegiano 1986; Aracne 2010). For *S. phleoides*, there are no antecedents in relation to its reproductive system and fertility (proportion of flower-forming fruits).

To provide information on the reproductive biology of *S. phleoides*, we (1) described the flowering phenology at population, inflorescence and spikelet level, and cleistogamy expression; (2) evaluated the breeding system through controlled pollinations; and (3) verified apomixis evidence through embryological studies.

Materials and methods

Studied species

Sporobolus phleoides is a caespitose, perennial, warm-season grass that reaches 50–100 cm tall. The inflorescences are spiciform panicles, 7–20 cm long and 0.3–0.4 cm wide, tightly contracted, densely flowered, truncate or rounded at the ends, and the branches that bear the spikelets are appressed or fused to the main axis. The spikelets are single–flowered, 1.6–2 mm long, disarticulating above the glumes at maturity; glumes are shorter than or equal to the palea and lemma. The anthecium bears a bisexual floret, with two lodicules and three stamens whose anthers reach 0.4–0.6 mm long. The fruit is a modified caryopsis with a free pericarp, typically swelling and mucilaginous when wet. The utricle is ellipsoid, 1.5 mm long, and light brown. (Parodi 1928; Steibel et al. 1997; Peterson et al. 2001; Denham 2012).

Plant material

Plants of *S. phleoides* used in this study originated from seeds collected from different locations in Argentina (Table 1). Voucher specimens were identified by a catalogue number (ID) and deposited in the Herbarium of the Facultad de Ciencias Agrarias-Universidad Nacional del Litoral (FCA-UNL) Esperanza, Santa Fe, Argentina (SF). These plants were grown in the experimental field of the FCA-UNL (31°27′04.7″S; 60°56′57.9″W). Each experimental population consisted of 20 plants arranged in a completely randomised design.

Phenological observations

Phenological observations were made during the flowering season from October 2012 to July 2013. At population level, dates of the beginning and end of flowering were recorded daily in ten randomly selected plants from each population. “Flowering beginning” was considered as the time at which the plant had at least one inflorescence with one spikelet at anthesis. Plants in which >50 % of the spikelets in the late-flowering inflorescences had open florets were considered as having “finished flowering”.

At the inflorescence level, flowering of the second-earliest fertile tiller was followed in ten randomly chosen plants of each population ($n = 10$ per population). The direction and duration of flowering were observed for each inflorescence.

At the spikelet level, phenological changes (shape, colour, timing and mode of anther opening, beginning of stigma receptivity, pollen release, and post-fertilisation changes) were recorded in 20 spikelets from five randomly chosen plants of each population ($n = 100$ per population).

Stigma receptivity

Flowering stages were identified as follows: (1) closed florets, near anthesis; (2) florets before fertilisation, whose anthers still remained closed; and (3) florets whose stigmas remained turgid after fertilisation. For each of these stages, ten florets from five plants per population were employed ($n = 150$ stigmas per population). Receptivity of the stigmas was determined by applying a solution of 6.6 mL water, 1.4 mL guaiacol, and 2 mL hydrogen peroxide (3 %) to the stigmas (adapted from Tel-Zur and Schneider 2009). Stigmas were considered receptive if they showed vigorous bubbling and colour change from white to orange-brown.

Pollen/ovule (P/O) ratios and pollen fertility

In ten randomly chosen plants of each population, nine unopened anthers per plant were collected from three florets ($n = 90$ anthers per population) and processed following the techniques proposed by Dafni (1992) to obtain counts of number of pollen grains per floret. These counts were used to compute P/O ratios (Cruden 1977). In five randomly chosen plants from each population, pollen was

Table 1 Plant populations of *Sporobolus phleoides*, their origins (province and locality), and voucher specimen IDs used in this study

Population ID[a]	Province	Locality	Lat.	Long.
8028	La Rioja	Dept. Gral. Belgrano	30°38′24.7″	65°35′40.2″
7610	Córdoba	Dept. Tulumba, Salinas Grandes	29°51′0.01″	64°40′10.9″
8415	Santa Fe	Dept. San Cristóbal	30°01′22.8″	61°16′12.2″
8452	Santa Fe	Dept. 9 of Julio	28°09′55.4″	61°44′19.8″
8393	San Luis	Dept. Gral. Pedernera	33°37′36.0″	65°26′4.4″
9722	Santa Fe	Dept. San Cristobal, in R. N. M. "El Fisco"	30°09′55.5″	61°00′23.3″

[a] ID identification number of the voucher specimen housed in the SF herbarium

collected from five florets per plant ($n = 25$ flowers per population) and immediately mixed. Their fertility was assessed from four samples of 200 pollen grains by determining the percentage of stainable per flower pollen in Lugol (2 % iodine–potassium iodide solution) (Scrivanti et al. 2009).

Percent of cleistogamy

The cleistogamy percentage was determined at the start of flowering for the period of November–December 2013. The percentage was calculated in 150 basal and 150 apical spikelets of five inflorescences from five randomly chosen plants per population ($n = 1500$ spikelets per population). The spikelets were considered cleistogamous following the criteria adopted by Rosengurt (1984). The cleistogamy percentage was defined as the number of cleistogamous spikelets divided by the total number of chasmogamous and cleistogamous spikelets (Clay 1983).

Breeding system

The breeding system was evaluated through self- and open-pollination treatments. Self-pollination was ensured by two methods: (1) inflorescences of randomly chosen plants ($n = 5$ per population) were covered with cheesecloth bags before anthesis and (2) inflorescences of randomly chosen plants ($n = 5$ plants per population) were isolated at 600 m from conspecific pollen source before anthesis. Open pollination (control) was guaranteed at the field where ($n = 5$ per population) inflorescences were screened. Seed set (fruit/flower ratio) was used to compare the treatments. For each plant, three samples of 50 spikelets from the upper and basal sectors of an inflorescence ($n = 300$ spikelets per inflorescence) were analysed. Thus, each population was represented by five randomly chosen plants ($n = 1500$ spikelets per population).

Embryological analyses

For embryological analyses, five inflorescences at different stages of development from three plants of each population ($n = 15$ inflorescences per population) were fixed in formalin, acetic acid, and alcohol (Ruzin 1999) and stored in 70 % ethanol. The dissected material was treated with 20 % hydrofluoric acid for 48 h to dissolve silica. The silica-free material was then dehydrated in an alcohol–xylol series and embedded in paraffin wax (D'Ambrogio de Argüeso 1986). The sections were cut with a rotary microtome at 7–9 μm thickness and stained with Safranin and Fast Green (D'Ambrogio de Argüeso 1986).

Statistical analyses

Duration of flowering at the population and inflorescence levels, percentage of receptive stigmas, pollen viability percentage, P/O rates, seed set among breeding systems treatments and cleistogamy percentage were analysed using analysis of variance (ANOVA), and treatments were compared using the Tukey test ($p < 0.05$). Results were presented as means and standard deviations. Data normality and homogeneity of variance were tested with Wilk–Shapiro and Bartlett tests, respectively. These analyses were performed with the statistical software package InfoStat (2011 version) (Di Rienzo et al. 2011). Differences in seed set production among different pollination treatments and populations were analyzed by the adjustment of general lineal models with the *lme* function of the *nlme* package (Pinheiro et al. 2011) of the R statistical language (R Development Core Team 2011), using the interface provided by InfoStat (Di Rienzo et al. 2011).

Results

Phenological observations

At the population level, flowering was homogeneous and lasted 206 ± 51 day ($F = 1.40$; $p = 0.20$), exhibiting a clear overlap between plants of different populations. Flowering began in early October 2012 and lasted until July 23, ending with the appearance of the first frost in early winter with 75 % of plants flowering.

Flowering lasted approximately 12 ± 1.60 day in inflorescences from all populations ($F = 1.10$; $p = 0.40$). The direction of flowering in the panicle was basipetal, beginning at the apex and continuing to the base. During the flowering period, a large number of spikelets in simultaneous anthesis were found, even in the same sector of the inflorescence (Fig. 1a). Spikelets in anthesis were registered since 7 a.m. to 18 p.m. during long days. All panicles presented chasmogamous and cleistogamous spikelets.

Before anthesis, most of the mature anthers turned pink and the stigmatic branches were already receptive. Within chasmogamous spikelets were found fully open florets (Figs. 1c, 2d) and partially open florets (Fig. 2d). For the fully opened floret, the anthesis started with the complete separation of palea, lemma and glumes, which allowed the total exposure of the stigmatic branches (Fig. 2b). In the meantime the staminal filaments were elongated leaving the anthers outside of the anthecium and above the stigmatic branches, process that took 30–40 min since the opening of anthecium (Fig. 2c). Over the next 20 min, the three anthers were opened longitudinally. This allowed the pollen to be released, falling on the stigmatic branches of the same floret or of the surrounding florets, while some were retained still on the anther. Then, anthers immediately changed their colour from white or pink to slightly yellowish or purplish.

In the partially open florets, stigmatic branches and stamens were largely retained inside the anthecium. The growth of the staminal filaments pushed the anthers towards the stigmatic branches (Fig. 2d) whereby the pollen was released directly on the stigmatic branches. However, it was also observed that some pollen released outside the floret.

In both cases, stigmatic branches progressively lost their turgidity and turned whitish after fertilisation. This process took 10 min in flowers where the anthers were nearer to the stigmatic branches (partially open florets) and more than 300 min in those whose anthers were more distant (fully open florets).

In cleistogamous spikelets, the glumes of the anthecium remained closed. The staminal filaments pushed the anthers towards the stigmatic branches, and pollen shed occured within the anthecium (Fig. 2e). Anthers and stigmatic branches were retained inside the anthecium until fruit formation (Fig. 1d). These spikelets were more frequent at the base of the inflorescence (Fig. 1b). At maturity, the pericarps were moistened and released the utricles outside the spikelets.

Observations across histological sections showed that both fully and partially open florets (chasmogamous) and fully closed (cleistogamous) ones, were morphologically similar.

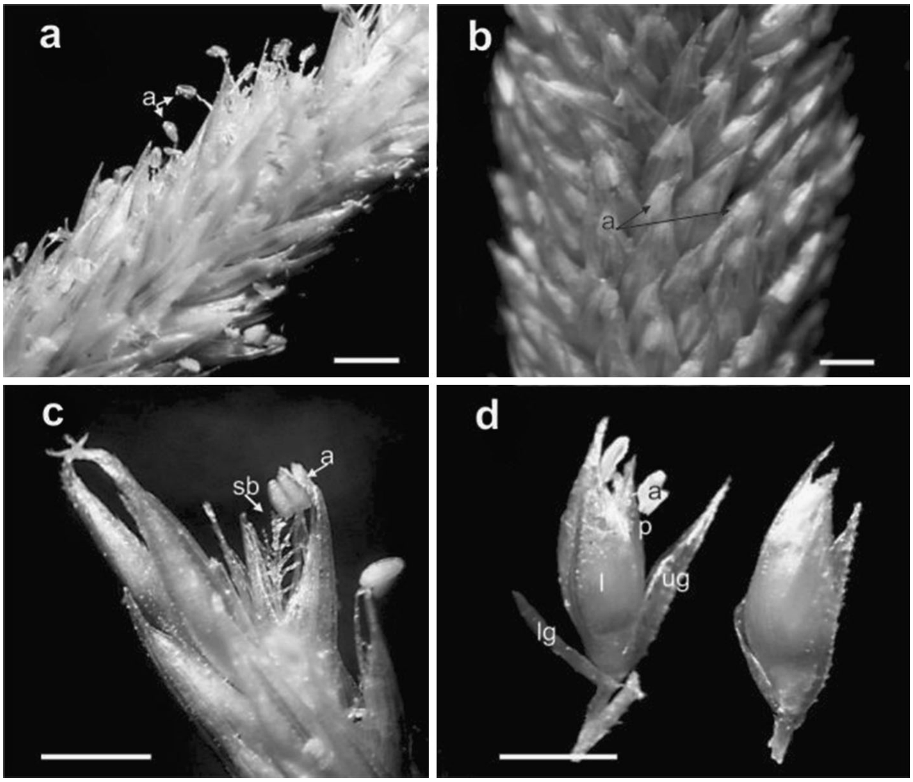

Fig. 1 Inflorescence and spikelet details of *Sporobolus phleoides*. **a** Chasmogamous spikelets in the *upper part* of the inflorescence with anthers outside florets; **b** Cleistogamous spikelets in the basal section of the inflorescence with anthers inside florets; **c** Fully open floret of a chasmogamous spikelet with anthers and stigmatic branches outside florets; **d** Immature fruit from chasmogamous (*left*) and cleistogamous (*right*) spikelets of *S. phleoides*. *a* anther, *l* lemma, *lg* lower glume, *p* palea, *sb* stigmatic branches, *ug* upper glume. *Bar* 1 mm

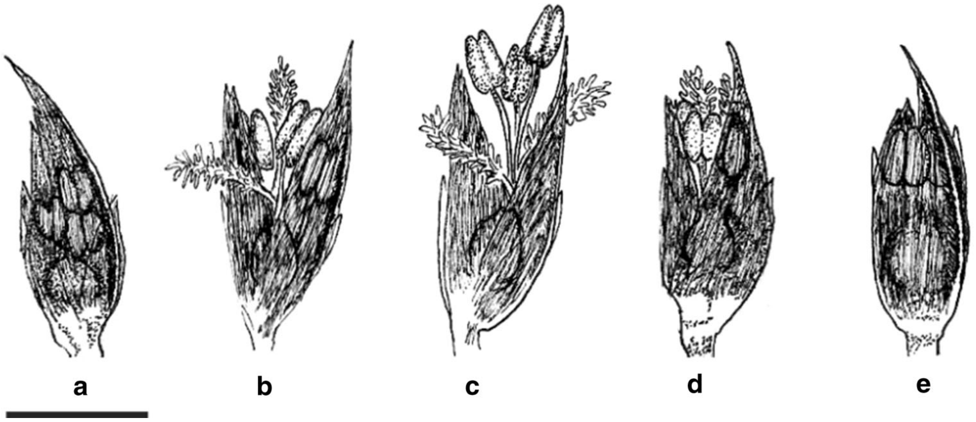

Fig. 2 Spikelets of *Sporobolus phleoides* **a** spikelet with a still-closed anthecium (stigmatic branches already receptive); **b** spikelet at start of anthesis, with complete separation of the palea, lemma, and glumes (stigmatic branches outside the floret, and anthers still inside); **c** Spikelet with a fully open floret (filament elongation allows anthers to place outside the anthecium and over the stigmatic branches); **d** Spikelet with a partially open floret (stigmatic branches and anthers are in contact); **e** Cleistogamous spikelet with closed floret. *Bar* 1 mm

Stigma receptivity

The receptivity of stigmatic branches started when florets were still closed and remained as such until their loss of turgidity. The receptivity lasted 40–360 min, depending on the degree of the floret openness, as mentioned previously. No significant differences between populations with respect to the duration of the stigmatic receptivity were found ($F = 1.40$; $p = 0.20$).

Pollen/ovule ratios and pollen fertility

The P/O ratios between populations showed significant differences ($F = 2.80$; $p = 0.04$), although they were small. The population 7610 had the highest proportion of pollen grains per ovule (314.90 ± 42.30), and the lowest (226.10 ± 16.70) was obtained in the 8415. In the remaining populations, the ratios obtained were not significantly different (Table 2). The viability of the pollen grains was 97.00 ± 2.10 %, with no significant differences between populations ($F = 0.90$; $p = 0.50$).

Percentage of cleistogamy

The percentage of cleistogamous spikelets averaged 23 ± 13 % of the total number of spikelets of the panicle for all populations tested ($F = 2.40$; $p = 0.06$). However, significant differences between basal (31 ± 12 %) and apical (15 ± 7 %) sectors of the inflorescences were observed ($F = 39.70$; $p < 0.01$) (Fig. 3). Fruit production was observed in virtually all the cleistogamous spikelets.

Breeding system

All plants from the analysed populations showed high percentages of seed set in an average between 95.01 ± 2.80 and 99.00 ± 2.70 %, indicating self-compatibility (Table 2). There were no significant differences in percentages of seed set between the conditions of self-pollination and open-pollination ($F = 1.10$; $p = 0.30$), or both sectors of the inflorescence ($F = 3.80$; $p = 0.06$). However, seed set was variable among populations ($F = 17.30$; $p < 0.01$). The highest percentage was obtained in the population 8452 with a mean of 98.67 ± 1.30 % and the lowest in the population 9722, with a mean of 96 ± 0.10 % for all evaluated treatments.

Embryological analyses

All studied plants showed a single megagametophyte per ovule. An archesporial cell was differentiated from the nucellus tissues and functioned as the megaspore mother cell (Fig. 4a). This was divided by meiosis, resulting in four haploid megaspores arranged in T-shaped tetrads (Fig. 4b). The chalazal megaspore turned functional, while the remaining three degenerated. This functional megaspore, through three successive mitotic divisions accompanied by wall formation, gave rise to a female gametophyte composed of two synergid cells, one egg cell, two polar nuclei, and three antipodal cells (Fig. 4c–f). Antipodes proliferated in mature gametophytes (Fig. 4g–i).

Discussion

The results indicate that *S. phleoides* reproduces sexually, is mainly autogamous, and combines chasmogamy with cleistogamy in the same inflorescence.

As in those species that produce cleistogamous and chasmogamous flowers at the same time in the same individual, it could be considered that *S. phleoides* has a

Table 2 Pollen/ovule (P/O) ratio, pollen fertility, and seed set in different pollination treatments of analysed populations of *Sporobolus phleoides*

Populations	Number of pollen grains per ovule (P/O)	Fertility of pollen grains (%)	Seed sets (%)		
			Self-pollination (isolated)	Self-pollination (covered)	Open pollination
8028	272.2 ± 57.2ab	96.50 ± 1.3	97.17 ± 2.0cd	96.50 ± 4.7cd	95.10 ± 2.8cd
7610	314.9 ± 42.3b	95.75 ± 30	98.17 ± 1.5b	96.90 ± 3.0b	97.60 ± 2.7b
8415	226.1 ± 16.8a	98.50 ± 1.9	96.17 ± 4.3b	98.60 ± 2.3b	98.20 ± 1.9b
8452	257.1 ± 36.4ab	97.75 ± 2.2	97.33 ± 5.3a	99.00 ± 1.0a	99.00 ± 2.7a
8393	265 ± 37.7ab	96.50 ± 1.3	97.33 ± 3.9bc	97.70 ± 1.2bc	97.10 ± 2.4bc
9722	255.4 ± 32.2ab	97.25 ± 2.5	97.67 ± 3.3d	95.00 ± 3.5d	95.70 ± 2.1d

Means with common letter are not significantly different ($p \leq 0.05$)

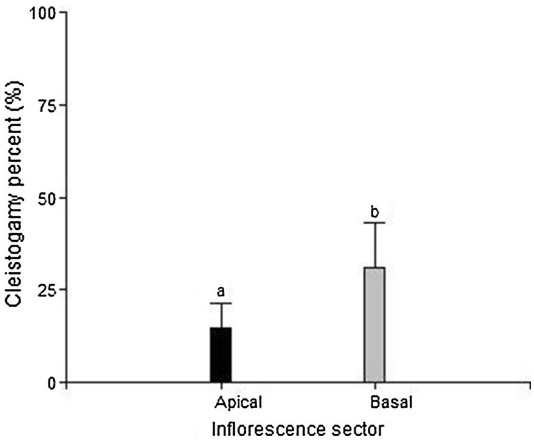

Fig. 3 Mean of cleistogamy percent (%) and standard deviation in apical and basal sectors of *Sporobolus phleoides* inflorescences during the observation period (November–December 2013). Means with common letters are not significantly different ($p \leq 0.05$)

mixed breeding system (Lloyd 1984; Schoen 1984; Redbo-Torstensson and Berg 1995; Masuda et al. 2001; Cheplick 2007). On this reproductive system, the balance between selfing and cross-pollination depends on the relative proportion of the two types of flowers, which is markedly plastic in most grasses (Campbell 1982; Clay 1983; Cheplick 1995, 2007). This is subject on how cleistogamy is affected. For many *Sporobolus* species, cleistogamous spikelets are enclosed in leaf sheaths at the time of fertilization and its florets may not open due to modified floral parts or perhaps simply mature precociously (Parodi 1928; Riggins 1977; Doyon and Dore 1967). This can encompass just a part or the totally the panicle and may be the result of an environmental response (Campbell et al. 1983). In *S. phleoides* we found that cleistogamous spikelets are exposed at the fertilization moment and they occur in a simultaneous way with their chasmogamous counterparts along the entire length of the panicle, with higher number at the basal sector. A similar situation was registered for *S. indicus* (Astegiano 1986) but in this case, cleistogamous spikelets are only on the upper part of the inflorescence. Although the study of the mechanism that prevents the opening of florets is not addressed in this work, it can be discarded that the cleistogamy in *S. phleoides* is resulting from any structural modification due to the isomorphy of both kinds of spikelets. This was also mentioned for *S. indicus*, and it was proved that florets remain unopened because of lodicule failure (Astegiano 1986). This type of cleistogamy often goes undetected, and represents one of the least frequent forms of cleistogamy on grasses (Campbell et al. 1983).

The relative proportion of the two kinds of flowers, and consequently, the percentage of their derived seeds, can also vary among populations of the same species (Campbell 1982, Clay 1983, Cheplick 2007, Culley and Klooster 2007). However, populations studied in this work show no consistent differences among their cleistogamy percentages, reaching approximately a 23 % despite their different origins. This percentage is closely linked to the reproductive system since cleistogamous and chasmogamous seeds usually differ in traits related to offspring fitness (Campbell et al. 1983; Lloyd and Schoen 1992; Oakley and Winn 2008). Because the environment, ontogeny, plant size and phenological stages can also affect the balance between cleistogamy and chasmogamy (Cheplick 2007), further studies must be conducted to better understand the reproductive strategy in this specie.

While cleistogamy guarantees a certain percentage of self-pollinating seeds, we propose that seed set in *S. phleoides* occurs primarily by autonomous selfing in chasmogamous florets. Observed traits like short floral life spans, small physical distance between male and female functions, and low P/O ratios are typical of highly selfing plants (Sicard and Lenhard 2011; Kalisz et al. 2012; Duncan and Rausher 2013). Even a certain amount of self-

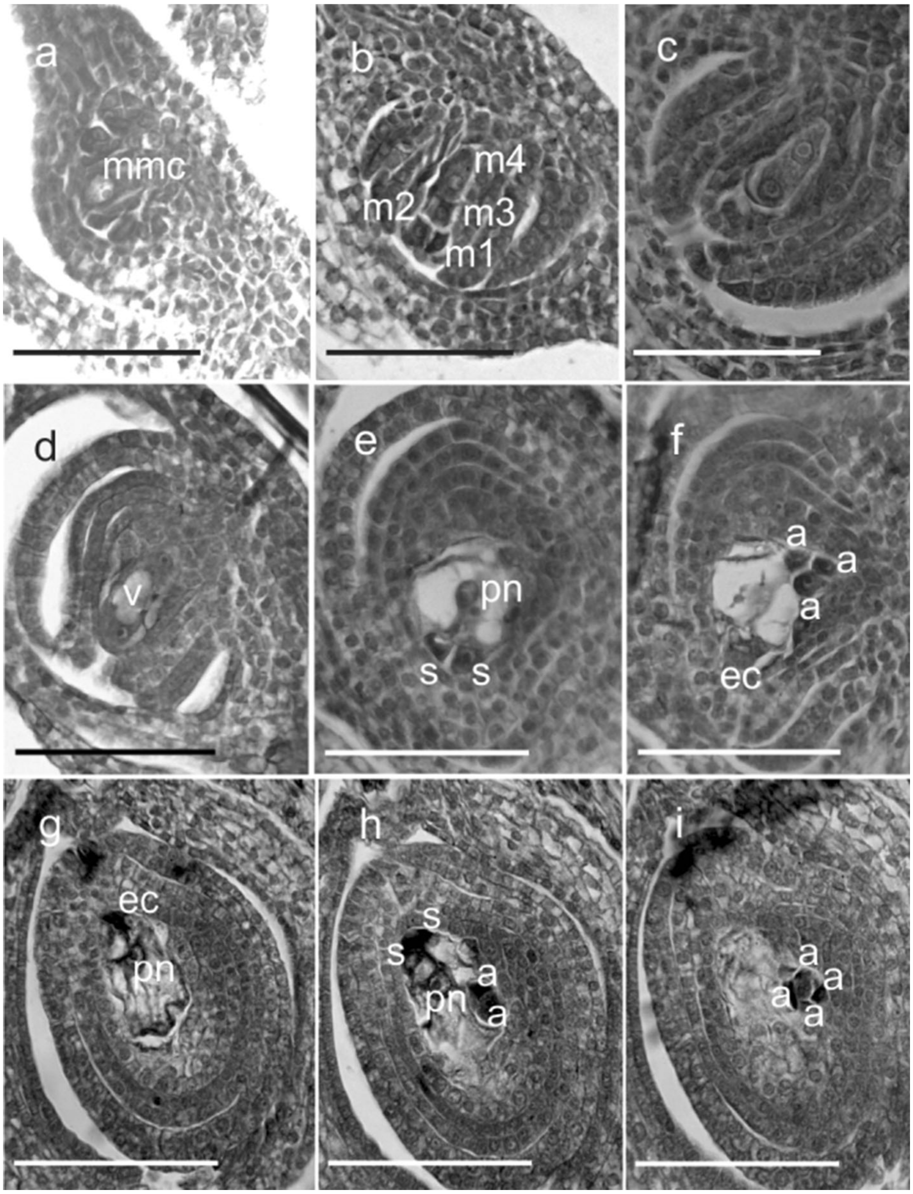

Fig. 4 Photomicrographs of *Sporobolus phleoides* ovules. **a** Megaspore mother cell; **b** T-shaped megaspore tetrad; **c** First mitotic caryocinesis of the viable megaspore; **d** Two-nucleate embryo sac with a central vacuole; **e**, **f** *Polygonum*-type megagametophyte with an egg cell, two polar nuclei, two synergids, and three antipodal cells; **g–i** Mature megagametophyte with a cluster of antipodal cells. *a* antipodal cells, *ec* egg cell, *m1*, *m2*, and *m3* megaspores, *m4* functional megaspore, *mmc* megaspore mother cell, *pn* polar nuclei, *s* synergid cell, *v* vacuole. *Bar* 50 µm

pollination by geitonogamy may be carried out spontaneously. This is a very common phenomenon in self-compatible species with compact inflorescences and profuse flowering (Cruden and Hermann-Parker 1977; Lloyd and Schoen 1992; Barrett 2002, 2003), especially if male and female phases are constantly overlapping (Lloyd and Schoen 1992). These mechanisms may ensure the high seed set reported in all evaluated populations of *S. phleoides*, which exceed the indicated values by Sutherland and Delph (1984) for self-compatible species. Thus, a high reproductive efficiency is obtained in the production of seeds and low levels of abortion of ovules and seeds.

In extreme environments where *S. phleoides* inhabits, high selfing rates present reproductive advantages, such as preserving gene combinations that confer high fitness in a local environment (Baker 1965; Stebbins 1957; Campbell et al. 1983; Richards 1986) and ensuring seed production when mates are scarce (reproductive assurance) (Baker 1955; Lloyd 1965; Inouye et al. 1996; Busch 2011; Cheptou 2012). In mixed breeding system species, gene flow is still maintained due to the cross-pollination potential of the chasmogamous flowers (Knight and Waller 1987; Culley and Klooster 2007).

While agamospermy was indicated by Smith-White (1988) for a triploid race of *S. virginicus*, the megagametogenesis and the megasporogenesis in *S. phleoides* follow the typical pattern of grasses with sexual reproduction systems, being very similar to the process described in *S. indicus* (Astegiano 1989). No irregularities in megasporogenesis or other indications of apomixis were recorded. At maturity, the embryo sacs show a Polygonum-type structure, typical on Poaceae (Anton and Cocucci 1984; Johri et al. 1992).

The information provided on the reproductive biology of this endemic halophytic species of Argentina will be useful to preserve genetic variability, to adequately plan their gene bank collection, and for the development of future work on improving forages.

Acknowledgments Thanks to Dr. DC Castro and to Mg. JM Zabala for his help with the statistical analysis of data; to Dr. MA Richard and Dr. MM Gotelli for helping with the English revision. Financial support for this research was provided by the Universidad Nacional del Litoral and the Consejo Nacional de Investigaciones Científicas y Técnicas.

References

Anton AM, Cocucci AE (1984) The grass megagametophyte and its possible phylogenetic implications. Pl Syst Evol 146:117–121. doi:10.1007/BF00984058

Aracne M (2010) Efecto de la polinización libre y la autopolinización en el éxito reproductivo pre-emergente y la germinación en poblaciones de *Sporobolus indicus* (Poaceae). Tesis de grado, Universidad Nacional del Litoral, Santa Fe

Aronsen JA (1989) Haloph, a data base of salt tolerant plants of the world. University of Arizona, Tucson

Astegiano ME (1986) La cleistogamia y casmogamia en *Sporobolus indicus* (Poaceae). Kurtziana 18:69–76

Astegiano ME (1989) Biología reproductiva de *Sporobolus indicus* (Poaceae) en relación a la interacción gametofito-esporofito. Kurtziana 20:65–94

Baker HG (1955) Self-compatibility and establishment after 'long distance' dispersal. Evolution 9:347–348. http://www.jstor.org/stable/2405656

Baker HG (1965) Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL (eds) The genetics of colonizing species. Academic Press, New York, pp 147–172

Barrett SCH (2002) Sexual interference of the floral kind. Heredity 88:154–159. doi:10.1038/sj.hdy.6800020

Barrett SCH (2003) Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. Philos Trans Ser B 358:991–1004. doi:10.1098/rstb.2003.1301

Busch JW (2011) Demography, pollination, and Baker's law. Evolution 65(5):1511–1151. doi:10.1111/j.1558-5646.2011.01224.x

Campbell CS (1982) Cleistogamy in Andropogon L. (Gramineae). Amer J Bot 69:1625–1635. http://www.jstor.org/stable/2442917

Campbell CS, Quinn JA, Cheplick GP, Bell TJ (1983) Cleistogamy in grasses. Annual Rev Ecol Syst 14:411–441. http://www.jstor.org/stable/2096980

Cheplick GP (1995) Plasticity of seed number, mass, and allocation in clones of the perennial grass *Amphibromus scabrivalvis*. Int J Pl Sci 156:522–529. http://www.jstor.org/stable/2475071

Cheplick GP (2007) Plasticity of chasmogamous and cleistogamous reproductive allocation in grasses. Aliso 23:286–294. doi:10.5642/aliso.20072301.23

Cheptou PO (2012) Clarifying Baker's Law. Ann Bot (Oxford) 109:633–641. doi:10.1093/aob/mcr127

Clay K (1983) Variation in the degree of cleistogamy within and among species of the grass *Danthonia*. Amer J Bot 70:835–843. http://www.jstor.org/stable/2442935

Connor HE (1979) Breeding systems in the grasses; a survey. New Zealand J Bot 17:547–574. doi:10.1080/0028825X.1979.10432571

Connor HE (1981) Evolution of reproductive systems in the Gramineae. Ann Missouri Bot Gard 68:48–74. http://www.jstor.org/stable/2398810

Cruden RW (1977) Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31:32–46. http://www.jstor.org/stable/2407542

Cruden RW, Hermann-Parker SM (1977) Temporal dioecism: an alternative to dioecism. Evolution 31:863–866. http://www.jstor.org/stable/2407448

Culley TM, Klooster MR (2007) The cleistogamous breeding system: a review of its frequency, evolution, and ecology in angiosperms. Bot Rev 73:1–30. doi:10.1663/0006-8101(2007)73[1:TCBSAR]2.0.CO;2

Dafni A (1992) Pollination ecology: a practical approach. Oxford University Press, New York. doi:10.1046/j.1420-9101.1993.6050776.x

D'Ambrogio de Argüeso A (1986) Manual de técnicas en histología vegetal. Hemisferio Sur, Buenos Aires

Denham SS (2012) *Sporobolus* R. Br. In: Zuloaga FO, Rúgolo ZE, Anton AM (eds) Flora vascular de la República Argentina. Monocotyledoneae, Poaceae: Aristidoideae a Pharoideae. Graficamente Córdoba, pp 208–218

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2011) InfoStat versión 2011. Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar

Doyon D, Dore WG (1967) Notes on the distribution of two grasses, *Sporobolus neglectus* and *Leersia virginica*, in Quebec. Can Field Nat 81:30–32

Duncan TM, Rausher MD (2013) Evolution of the selfing syndrome in *Ipomoea*. Front Plant Sci 4(301):1–8. doi:10.3389/fpls.2013.00301

Engstrom B (2004) *Sporobolus compositus* var. *compositus* (Tall Dropseed). Conservation and research plan for New England. New England Wild Flower Society, Framingham, Massachusetts, USA

Febles G, Padilla C, Achan G (2010) Estudios acerca de la reproducción agámica en *Sporobolus indicus.* Revista Cubana de Ciencia Agrícola 44(2):185–187. http://www.redalyc.org/pdf/1930/193015662015.pdf

Fryxell PA (1957) Mode of reproduction of higher plants. Bot Rev 3:135–233. doi:10.1007/BF02869758

Heslop-Harrison Y, Heslop-Harrison JS (1996) Lodicule function and filament extension in the grasses: potassium ion movement and tissue specialization. Ann Bot (Oxford) 77:573–582. doi:10.1093/aob/77.6.573

Inouye K, Maki M, Masuda M (1996) Evolution of Campanula flowers in relation to insect pollinators on islands. In: Lloyd DG, Barrett SCH (eds.) Floral biology: studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York, pp. 377–301. doi:10.1007/978-1-4613-1165-2_14

Johri BM, Ambegaokar KB, Srivastava P (1992) Comparative embryology of angiosperms. Springer-Verlag, New York

Joshi AJ, Malia BS, Hinglagiaa H (2005) Salt tolerance at germination and early growth of two forage grasses growing in marshy habitats. Environm Exp Bot 54:267–274. doi:10.1016/j.envexpbot.2004.09.005

Kalisz S, Randle A, Chaiffetz D, Faigeles M, Butera A, Beight C (2012) Dichogamy, outcrossing rates and selfing syndrome in mixed-mating *Collinsia*. Ann Bot (Oxford) 109:571–582. doi:10.1093/aob/mcr237

Khan MA, Gul B (2008) Halophyte seed germination. In: Khan MA, Weber DJ (eds) Ecophysiology of high salinity tolerant plants. Springer, Netherlands, pp 11–30

Knight SE, Waller DM (1987) Genetic consequences of outcrossing in the cleistogamous annual *Impatiens capensis*. I Population genetic structure. Evolution 41:969–978. http://www.jstor.org/stable/2409185

Liphschitz N, Waisel Y (1974) Existence of salt glands in various genera of the gramineae. New Phytol 73:507–513. doi:10.1111/j.1469-8137.1974.tb02129.x

Lloyd DG (1965) The evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). Contr Gray Herb 195:3–134. http://www.jstor.org/stable/41764680

Lloyd DG (1984) Variation strategies of plants in heterogeneous environments. Biol J Linn Soc 21:357–385. doi:10.1111/j.1095-8312.1984.tb01600.x

Lloyd DG, Schoen DJ (1992) Self and cross-fertilization in plants. I. Functional dimensions. Int J Pl Sci 153:358–369. http://www.jstor.org/stable/2995676

Masuda M, Yahara T, Maki M (2001) An ESS model for the mixed production of cleistogamous and chasmogamous flowers in a facultative cleistogamous plant. Evol Ecol Res 3:429–439. http://www.evolutionary-ecology.com/abstracts/v03n04/ffar1246.pdf

Mc Gregor RL (1990) Seed Dormancy and Germination in the annual cleistogamous Species of *Sporobolus* (Poaceae). Trans Kansas Acad Sci 93(1–2):8–11. http://www.jstor.org/stable/3628122

Oakley CG, Winn AA (2008) Population-level and family-level inbreeding depression in a cleistogamous perennial. Int J Pl Sci 169(4):523–530. doi:10.1086/528752

Ortiz-Díaz JJ, Culham A (2000) Phylogenetic relationships of the genus *Sporobolus* (Poaceae: Eragrostideae) based on nuclear ribosomal DNA ITS sequences. In: Jacobs S, Everet J (eds) Grasses: systematics and evolution. CSIRO, Melbourne, pp 184–188

Parodi LR (1928) Revisión de las especies argentinas del género *Sporobolus*. Rev Fac Agr y Vet 6(2):115–168

Peterson PM, Soreng R, Davidse G, Filgueiras T, Zuloaga F, Judziewicz E (2001). Catalogue of New World grasses (Poaceae): II. Subfamily Chloridoideae. Contr US Natl Herb 41:1–255. http://www.jstor.org/stable/23493180

Peterson PM, Hatch S, Weakley A (2003) *Sporobolus* R. Br. Pp. In: Barkworth M, Capels K, Long S, Piep M (eds). Flora of North America Editorial Committee, Flora of North America. Oxford Univ. Press, New York, 25, pp 115–139

Peterson PM, Columbus T, Pennington S (2007) Classification and biogeography of New World grasses: chloridoideae. Aliso 23(1):580–594. doi:10.5642/aliso.20072301.43

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2011) nlme: linear and nonlinear mixed effects models. R Package Version 3.1-101

Redbo-Torstensson P, Berg H (1995) Seasonal cleistogamy: a conditional strategy to provide reproductive assurance. Acta Bot Neerl 44:247–256. doi:10.1111/j.1438-8677.1995.tb00783.x

Richards A (1986) Plant breeding systems. George Allen and Unwin Publisher, London

Riggins R (1977) A biosystematic study of the *Sporobolus asper* complex (Gramineae). Iowa State J Res 51(3):287–321

Rogers ME, Craig AD, Munns R, Colmer TD, Nichols PGH, Malcolm CV, Barrett-Lennard EG, Brown AJ, Semple WS, Evans PM, Cowley K, Hughes SJ, Snowball R, Bennett SJ, Sweeney GC, Dear BS, Ewing MA (2005) The potential for developing fodder plants for the salt-affected areas of southern and eastern Australia: an overview. Aust J Exp Agr 45(4):301–329. http://dx.doi.org/10.1071/EA04020

Rosengurt B (1984) Gramíneas cleistógamas del Uruguay. Bol Fac Agron Univ Rep Montevideo 134:1–28

Rosengurtt B, Arrillaga de Maffei BR (1961) Flores cleistógamas en gramíneas uruguayas. Bol Fac Agron Univ Rep Montevideo 57:1–12

Ruzin SE (1999) Plant Microtechnique and Microscopy. Oxford University Press, New York

Schoen DJ (1984) Cleistogamy in *Microlaena polynoda* (Gramineae): an examination of some model predictions. Amer J Bot 71:711–719. http://www.jstor.org/stable/2443368

Scrivanti LR, Norrmann GA, Anton AM (2009) Reproductive biology of South American *Bothriochloa* (Poaceae: Andropogoneae). Flora 204(9):644–650. doi:10.1016/j.flora.2008.09.005

Sicard A, Lenhard M (2011) The selfing syndrome: a model for studying the genetic and evolutionary basis of morphological adaptation in plants. Ann Bot (Oxford) 107:1433–1443. doi:10.1093/aob/mcr023

Smith-White AR (1988) *Sporobolus virginicus* (L.) Kunth in Coastal Australia: the reproductive behaviour and the distribution of morphological types and chromosome races. Austral J Bot 36:23–39. doi:10.1071/BT9880023

Stebbins GL (1957) Self-fertilization and population variability in higher plants. Amer Naturalist 91:337–354. http://www.jstor.org/stable/2458946

Steibel P, Rúgolo de Agrasar Z, Troiani H, Martínez O (1997) Sinopsis de las gramíneas (Gramineae Juss.) de la Provincia de La Pampa, República Argentina. Revista Fac Agron Univ Nac La Pampa 9:1–122

Sutherland S, Delph LF (1984) On the importance of male fitness in plants: patterns of fruitset. Ecology 65:1093–1104. http://www.jstor.org/stable/1938317

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/

Tel-Zur N, Schneider B (2009) Floral biology of *Ziziphus mauritiana* (Rhamnaceae). Sex Pl Reprod 22:73–85. doi:10.1007/s00497-009-0093-4

Watson L, Dallwitz MJ (1992). The grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. Version: 12th August 2014. http://delta-intkey.com

Weaver JE (1954) North American prairie. Johnsen Publishing Company, Lincoln

Wood JN, Gaff DF (1989) Salinity studies with drought-resistant species of *Sporobolus*. Oecologia 78:559–564. doi:10.1007/BF00378748