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B chromosomes and fertility in a native population of *Hymenachne amplexicaulis* (Poaceae: Panicoideae: Paspaleae)

Fabiana Eckers[b], Claudia B. Sorol[b], Julio R. Daviña[a], Ana I. Honfi[a,*]

[a] Programa de Estudios Florísticos y Genética Vegetal, Instituto de Biología Subtropical (IBS, CONICET-UNaM), Facultad de Ciencias Exactas Químicas y Naturales (FCEQyN), Universidad Nacional de Misiones, Rivadavia 2370, 3300, Posadas, Misiones, Argentina

[b] Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones (UNaM), Posadas, Misiones, Argentina

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ABSTRACT

Fertility is a measure of reproductive success, defined as the capacity of an individual to reproduce and generate offspring, and has been identified as an important trait correlating to the invasive ability of a species. *Hymenachne amplexicaulis* (Rudge) Nees is a grass native to South America of high bromatological quality, making it a species with good forage potential. In the places where it was introduced it is now considered an invasive weed due to its dominance over native vegetation. In this work, individuals of *H. amplexicaulis* were analyzed from a native population in its southernmost native range, situated on Yacyretá reservoir dam. All individuals show a diploid cytotype with two B chromosomes ($2n = 2x = 20 + 2B$). This is the first chromosome count recording B chromosomes for the genus, and a new cytotype for the species. The karyotype was found to be symmetrical and unimodal, composed by 20 metacentric chromosomes and two small B chromosomes. Pollen viability was tested at 90.6%, seed set production of population reaches 18.65%, and the viable seeds presented a germination power of 41%. The reproductive performance is less than expected with respect to the mean amount of spikelets that a single inflorescence possesses and notwithstanding optimal conditions of supply and availability of viable pollen for fecundation. Germination test indicate good seed quality and viability in native range. In *H. amplexicaulis* effective fertility is lower than its potential fertility, but it is not an indicator of absence of invasive threat. Reproductive performance of B host plants indicates sufficient invasive potential.

1. Introduction

Fertility is a measure of reproductive success, defined as the capacity of an individual to reproduce and generate offspring. Once the floral structures have been developed, there are at least three crucial genetically determined processes whereby the fertility of an individual may be defined: first, at sporogenesis and gametogenesis, wherein proper meiosis is required to produce viable and chromosomally balanced spores and gametes. Then, during pollination, at which point the pollen tube must successfully develop in the style and reach the embryo sac, requiring complete pollen-pistil compatibility; lastly, at fecundation, the embryo and endosperm tissues must developed in harmony with the maternal tissues to produce a functional seed. If any one of these three systems is altered, and the post-zygotic survival of the progeny is not secured, consequently, the fertility of the individual will be affected (Lloyd, 1979, 1980; Grant, 1989). In plants, the number and size of seeds constitute basic quantitative traits for female reproductive fitness (Braza et al., 2010), and they play a central role in the ecology and evolutionary genetics of a species. The maximum number of fruits that may be produced in a reproductive event depends on the number of female flowers, while the number of seeds is determined by the number of ovules within the gynoecium, which represent the potential reproductive capability of an individual plant. The fraction of this potential reproduction that is then transformed into fruits and seeds represents the real reproductive capacity (Stephenson, 1981). In the case of grasses, each spikelet contains one to several florets (or flowers), each floret contains a single unilocular ovary with one ovule, and in each reproductive event, the developed fruit is a caryopsis representing a single seed (Gould and Shaw, 1992). Fecundity is an effective and real trait of fertility, and refers to the number of offspring produced by an individual or population at a given time, as determined by genetic as well as environmental factors, and it is the principle indicator of reproductive success or fitness (Lloyd, 1979, 1980). The parameters of fertility and fecundity reflect the differences between the potential and real capability of the population to produce offspring. In the case of grasses, the potential fertility of a population can be estimated as the average number of spikelets per inflorescence or per individual, while the real fertility or fecundity is the average frequency by which the

* Corresponding author.
E-mail address: ahonfi@gmail.com (A.I. Honfi).

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individuals of a population actually produce offspring. Thus, for grasses the real fertility corresponds to the proportion of viable seeds produced per inflorescence or per individual.

Fertility has been identified as an important trait correlating to the invasive ability of a species (Burns et al., 2013) and basically, implies that more fecund individuals produce greater numbers of propagules, seeds, or clonal materials to the subsequent generation, and for this reason, more fecund individuals or species have more opportunities to colonize new sites (Westoby et al., 2002). It would seem intuitive to consider invasive species as more fecund and growing more rapidly than non-invasive species (Baker, 1965). The fertility of a plant might be affected by various factors, including those of chromosomal nature, i.e. structural rearrangements in heterozygous conditions (translocations and inversions), or lack of homologous pairing during meiosis, which fundamentally alters chromosomal segregation and cause gametic sterility (Grant, 1989). The presence of B chromosomes has been also related with variations in the fertility of its carriers. Supernumerary, accessory, or *B* chromosomes are expendable superfluous add-ons to those of the normal complement (named *A* chromosome set), and they do not pair nor recombine with these, behave irregularly and have non-Mendelian heredity (Camacho et al., 2000, Jones et al., 2008). The presence of B chromosomes can go unnoticed and produce no effect, or they can be detected in the phenotype of the carrier plant and seems to affect such characteristics as development rate, vigor, and fertility. The effect on these traits are variable, in low doses ranging from advantageous to unfavorable, whereas with higher numbers the result is always adverse (Jones, 1995). Randolph (1941) encountered for the first time that in corn, when many B chromosomes are found in the same nucleus, there is a marked reduction in vigor and fertility. Similar results have been found in different species by various authors (Müntzing, 1943; Bosemark, 1957). The effect of supernumerary chromosomes on the germination of seeds depends on the particular species, favoring germination in *Allium porrum* L., *A. Schoenoprasum* L., *Anthoxanthum alpinum* Á. Löve & D. Löve and *Picea glauca* (Moench) Voss, while the effects are neutral or detrimental in other studied species (Jones, 1995). Fertility is very sensitive to the presence of B chromosomes, and a wide range of its effects have been described from the genetic to demographic point of view. For example, in rye (*Secale cereale* L.) the presence of B chromosomes has been observed to diminish fertility, and this effect to increase with the number of supernumerary chromosomes present in a single nucleus, while the plants' vigor was seen to be neither positively nor negatively influenced (González-Sánchez et al., 2004).

Hymenachne P. Beauv. is a genus comprised of approximately ten species of pantropical grasses that are found in swampy environments, are perennials, and have decumbent stems which are radicant in the inferior nodes (Nicora and Rúgolo De Agrassar, 1987). The inflorescence is an open or contracted panicle with the spikelets including a potentially fertile lower lemma and a sterile upper lemma. In America, *Hymenachne* has four representative species: *H. amplexicaulis* (Rudge) Nees, *H. donacifolia* (Raddi) Chase, *H. grumosa* (Nees) Zuloaga and *H. pernambucensis* (Spreng.) Zuloaga, all are found in Argentina (Zuloaga et al., 2003).

Hymenachne amplexicaulis is a native species of South America, distributed from Mexico and the Antilles, to Argentina, Brazil, Paraguay, and Uruguay (Zuloaga et al., 2003). This heliophyte is found in low humid places, on the banks of rivers, streams, and lagoons, as well as alongside canals and reservoirs (Zuloaga et al., 1994). Phenologically, *H. amplexicaulis* primarily fructifies in summer, blooming in Paraguay from September to June (Zuloaga et al., 1994), in Brazil from January to October, and in northeast Argentina from October to April. It reproduces sexually by seeds, and vegetatively by stolons and propagules (Sellers et al., 2008).

The coexistence of sexual reproduction and vegetative propagation, assuming a limited supply of parental energy, is reconciled in many plants with an inverse correlation between the two reproductive processes, in such manner that abundant vegetative propagation is usually associated with scarce seed production, and vice versa (Grant, 1989). *Hymenachne amplexicaulis* is of interest as a forage, given its excellent bromatological quality, with average digestibility values ranging from 66 to 80%, and high protein content when analyzing the whole plant (15.8%), or 22.6% in the leaves and 9% in the stem (Wearne et al., 2010). Accordingly, due to these qualities it was introduced in the 1970's in Australia; where today is however considered an aggressive invader that dominates over native population in flood-pronelands and affects crops such as sugar cane (Csurhes et al., 1999). Also, *H. amplexicaulis* forms large dense clusters in seasonal fresh water marshes in Australia (Csurhes et al., 1999), and in northeastern Argentina it behaves as a macrophyte in riversand streams. Aquatic plants play an important role in aquatic ecosystems (Araya et al., 2013). However, excessive growth in freshwaters have both ecological and economic impacts, mainly because creates ecological changes in the macrophyte community composition, alters limnological parameters and water quality, and increase flood risks by obstructing river flow (Hussner et al., 2017).

Few chromosome records have been reported within the native range of *H. amplexicaulis*. Basic chromosome number of *Hymenachne* is $x = 10$ (Honfi et al., 1990). In America, the cytotype with $2n = 2x = 20$ chromosomes has been found in Costa Rica, Venezuela, Colombia and Brazil (Honfi et al., 1990 and references therein), and a count of $2n = 24$ chromosomes was also found for a sample of unknown origins (Enriquez-Quiroz et al., 2006).

Given the invasive nature of the species and the fact that its natural habitat has been altered (flood plains changes due to the dam and variation in rainfall regimes), the preliminary identification, of a natural population in southern Misiones, Argentina of *H. amplexicaulis* carrying a B chromosome, provide an excellent opportunity to analyze its reproductive performance, karyomorphological features and invasive potential.

2. Materials and methods

2.1. Plant material

In April 2014, samples were taken of a natural plant population (*Honfi 1709*) located near the mouth of Yabebiry Stream, a tributary of the Paraná River, located at Department of Candelaria, Province of Misiones, Argentina (S 27°17′15.6″, W 55°32′06″). The sampling site is located in the area of marshes and other environments associated with the upstream of Yacyretá Dam, whose surrounding zones can be characterized as being low banked, flood-prone fields, with the presence of sandy soil and sandbanks. The low river basin of the Yabebiry stream exhibits considerable development of flood plains and sporadic marshlands, and receives substantial allochthonous material from the bordering relictual forest (margin vegetation) and from terrestrial vegetation during flood pulse periods. Since the filling of the Yacyretá reservoir, the flood plain has grown, transforming into a lateral sub-reservoir with greater periods of the presence of water (Araya et al., 2013). These sub-reservoirs are affected and conditioned by fluctuations in the flow and depth of the river, and in the case of the Yabebiry stream, the period of low waters corresponds to February and March, while the high water period is from August to October (Araya et al., 2013).

Morphologically, the studied individuals of *H. amplexicaulis* belongs to a monomorphic population with decumbent stems at the base, which then ascend to a height of approximately 1.89 m, from the base of the shoot to the tip of the inflorescence, presenting roots in the lower nodes. The leaves are approximately 24×1.7 cm, flat. The inflorescences are cylindrical, terminal, contracted, 27.5 cm in length and 1.8 cm in width, with short ramifications of up to 4 cm in length. The spikelets are acuminate, lanceolate, unilaterally arranged and of 4×0.5 mm in length. The amount of inflorescences (panicules) per m^2,

vary by season and places according to river dynamics, from 4 to 6.

In order to carry out the cytogenetic studies, parts of the rhizomes of individuals randomly selected were transplanted in pots and cultivated with GROWMIX-PRO substrate, composed of *Sphagnum* peat moss of medium and fine fibers, vermiculite, lime calcite, lime dolomite, humidifying agents, sand and soil. Herbarium voucher specimens were deposited at the herbarium of the Universidad Nacional de Misiones (MNES), registered at the Sistema Nacional de Datos Biológicos (SNDB) of Argentina.

2.2. Fertility and germination studies

For the plant fertility studies, mature inflorescences where harvested prior to seed dispersion, placed in paper envelopes and kept at room temperature until dried, then stored until utilization. The inflorescences were harvested at random during the period of maximum fructification (summer's end) from plants separated each from the other by a distance of at least 2 m at the field. All seed samples were collected from within the same population. After collection, all seeds were stored in darkness (inside brown paper bags) in an air-conditioned room kept at a constant temperature of 25 °C until used in germination experiments.

Male fertility was assessed through measurement of the viability of pollen grains obtained from mature anthers of inflorescences in the field and fixed in a solution of absolute ethanol: lactic acid (5:1). The pollen grains were then dyed with carmine: glycerine (1:1) for 24 h. One thousand grains were analyzed, differentiating between those presenting completely coloured cytoplasm (viable), and those left uncoloured (non-viable). The percentage of viable pollen grains was calculated considering the number of stained grains with respect to the total analyzed.

The production of seeds was evaluated in natural conditions of open pollination, using mature inflorescences harvested in the field, taken at random, from different individual plants. Each inflorescence was examined with a stereoscopic microscope and the number of total spikelets in each inflorescence was recorded, differentiating between those with developed grains (caryopsis) from empty spikelets. For this purpose, spikelets were lightly squeezed with curved blunt tipped tweezers to detect the presence of a developed caryopsis. In the case of a caryopsis lacking full development, the glumes were removed and it was observed under stereoscopic microscope. The percentage of seeds at population level was evaluated based on the average values of seed set produced per inflorescence. The length and width of 50 seeds were selected at random, and the average and standard error were calculated.

In order to evaluate if the seed bearing inflorescences were harvested at the moment of optimal maturity, they were subjected to humidity content analysis, carried out in accordance with the high temperature stove method (130 ± 1 °C for 1 h), expressed in percentage, based on humid weight (Brasil, 2009) and calculating the difference in beginning and end weights according to the formula: HC = (initial weight-final weight)/(initial weight- weight of recipient). Seed mass was estimated based on the weight of 1000 seeds, by the method put forth in Rules for Seed Analysis (Brasil, 2009), where, measuring the weight of 8 samples of 100 seeds each, the weight of 1000 seeds is estimated, considering the median weights with a coefficient of variation less than 6 for dressed seeds, given that in this species glumes are persistent.

The germination trials were carried out by sowing the seeds on paper laid out on plastic trays in a germination chamber with a light period of 8 h and alternate temperature 25/35 °C. The number of germinated seeds was registered daily, considering as germinated those seeds presenting the emergence of the radicle. The variables of germination evaluated were *i*) germinating power (GP) calculated as the total seeds germinated divided by the total seeds sowed, *ii*) time in which 50% of the viable seeds had germinated (T50); and *iii*) the velocity of germination estimated by the germination velocity index GVI = $\Sigma\ (n_i/\Sigma t_i)$, where n_i is the number of germinated seeds in the time interval t_i, and Σt_i is the period from the sowing up to t_i (Maguire, 1962). Four repetitions of 100 seeds each were carried out. The data was analyzed statistically, estimating the average and standard deviation for each of the germination variables.

2.3. Cytogenetics

The determination of the chromosome number and ploidy level was carried out on root apical meristems pretreated with α-bromonaphtalene saturated solution for 3 h and then fixed in Farmer fixative solution (absolute ethyl alcohol: glacial acetic acid, 3:1). The meristems were dyed with conventional Feulgen staining, for which the root tips were hydrolyzed with HCl 1N for 10 min at 60 °C in a thermostatic bath and afterwards stained with Schiff Reactive (basic fuchsin). In order to increase the contrast in coloration, the meristems were macerated with 2% aceto-orcein. The karyotype was described according to the position of the centromere of each chromosome following the nomenclature of Levan et al. (1964), in four categories: metacentric (*m*), submetacentric (*sm*), subtelocentric (*st*), and telocentric (*t*) and they were arranged in decreasing order of length for each chromosome type. Ten optimal metaphases were selected to carry out a representative ideogram. The morphometric parameters measured were the length of the short (*s*) and long (*l*) chromosome arms, the total chromosome length (*c*), and the centromeric index (*i*) was calculated for each chromosome. Karyotype asymmetry was estimated using the asymmetric intrachromosomic (A_1) and interchromosomic (A_2) indexes of Romero Zarco (1986) and the categories of Stebbins (1971). For the observation and photography of the mitotic preparations, a Leica DML microscope, with DFC310 FX video camera and image processor, was used.

3. Results

3.1. Fertility and germination test

In the analysis of the pollen grains, out of a total of 1000 grains, 906 presented complete coloration, demonstrating a pollen viability of 90.6%. No morphological variations were observed in appreciable shape or form upon comparing the viable and unviable grains, which is to say that the pollen of *H. amplexicaulis* is homogenous with regards to size (Fig. 1).

A total of 12,617 spikelets of *H. amplexicaulis* were analyzed, of which 2353 had developed a caryopsis. On average, each inflorescence (panicule) has 3155 spikelets, of which 589 developed a caryopsis, indicating that the rate of seed production per inflorescence is 17.04% ± 2.97. Seed production on the population level, was 18.65% with a standard deviation of 5.25. The length – width measures of seeds

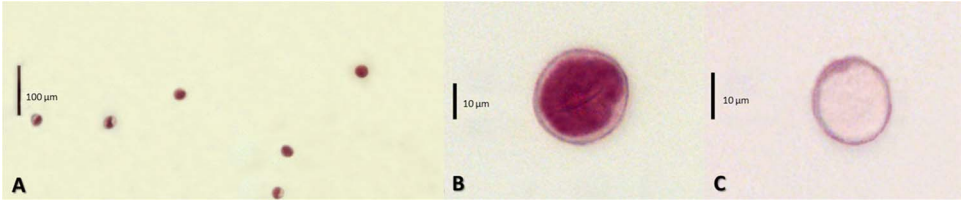

Fig. 1. Pollen grains of *Hymenachne amplexicaulis* stained with carmine-glycerine. A- Full view of pollen grains. Scale: 100 *µm*. B- Viable pollen grain. C- Non-viable (uncoloured) pollen grain. Scale of B and C: 10 *µm*.

Table 1
Seed performance and parameters of germination in seeds of B-chromosome host *Hymenachne amplexicaulis* ($2n = 2x = 20 + 2B$).

Mean Seed production	18.65% ± 5.25
Mean seeds formed per inflorescence	588 seeds
Weight of 1000 seeds	0.45 g
Humidity Content	13.123%
Germination power	41% (± 3.06)
VGI	10.54 ± 1.30
T50	5 days

VGI: index of Germination velocity. T50: Time elapsed when 50% of the seeds have germinated. SE: Standard error.

had an average of 3.97 mm ± 0.04 (length) x 0.98 mm ± 0.007 (width). The seed mass as measured by the estimated weight of a thousand seeds was 0.45 g, with a coefficient of variation (CV) of 3.05%. The humidity content of the seeds was 13.123% (Table 1).

The germination of the seeds of *H. amplexicaulis* began two days after being sown, reaching maximum strength on approximately the eighth day following the sowing (Fig. 2). The germinating power was 41% (standard deviation SD = 3.06), the IVG was 10.54 (SD = 1.30), and T50 was approximately five days. The experiments were ended 21 days after sowing (Table 1).

3.2. Chromosome studies

Cytologically, all the individuals of the analyzed population of *H. amplexicaulis* presented a diploid cytotype with two B chromosomes ($2n = 2x = 20 + 2B$), that were markedly smaller in size those of the A complement (Fig. 3A and B). This is the first time that a supernumerary chromosome condition for this species and genus is recorded. Also, up to the present, no chromosomal studies on this species had been made in native southern native range. The karyotype is composed of 20 small metacentric chromosomes and two B chromosomes (Fig. 4, Table 2). The unimodal karyotype composed of metacentric, symmetrical chromosomes, had 1A Stebbins' Category and values close to zero in the intra- as well as inter-chromosomical asymmetry indexes of Romero Zarco (Table 3). The B chromosomes presented a length of 1.00 µm and 0.91 µm, respectively, and were smaller than the smallest of the A complement (pair 10 *m* with 1.3 µm). The total length of the chromosome complement is 34.65 µm with an average chromosome length of 1.73 µm and a range of variation between 1.3 and 2.17 µm. The genome size increases from 34.65 µm to 36.56 µm, or 5.51%, with the presence of the B chromosomes

4. Discussion

Our study shed light on the reproductive performance of *H. amplexicaulis*, particularly in a native population where individuals carried two B chromosomes. Reproductive performance, evaluated by estimations of seed set production per inflorescence and germination of viable seeds, per individuals and at population level, is a biological parameter indicating the potential for a species to establish itself in an ecosystem. The production of seed set per inflorescence and per population encountered in its native range (17.04% ± 2.97 and 18.65% respectively) is much less than expected with respect to the mean amount of spikelets that a single inflorescence possesses (3155 spikelets), notwithstanding optimal conditions of supply and availability of viable pollen for fecundation. Others factors are influencing the seed performance. No clear data are available about seed set production analysis of *H. amplexicaulis* as invader or forage species that allow a good comparison with our results coming from a native population belonging of the southernmost range of the species. Wearne et al. (2010) report that recent experiments in Australia where the species is an invasive wetland grass, show that a single flower head producing in excess of 4000 seeds, and on average 26 ± 21 flower heads $m^{-2} y^{-1}$ are produced. Also, as an agronomic data, Hacker and Loch (1997) inform that at commercial seed production level, *H. amplexicaulis* produces a mean of 75 seed yield (kg/ha). The mechanisms of reproduction and dispersion of *H. amplexicaulis*, both sexual and vegetative, favor invasive behaviour, not only in adapting to changes in water depth by rapid elongation of its stalk, but also the increase in leaf area, and the production of adventitious roots (Sellers et al., 2008); although based on our results, in spite the 17.04% of seed set seems few, the amount of 589 seeds/inflorescence are sufficiently great to colonize the surrounding area of the mother plant (or floral culm). Seedlings are probably the main critical step in the invasive behaviour by sexual means, whose competition with old parent rhizomes determines the progeny survival rate. Periodical flooding and dynamic of Paraná River coasts, are also factors involved in this colonization step, reducing the population size and favoring new uninhabited patches. Standard germination test is an indicator of seed quality and viability which can be used to predict the field emergence. In a batch of fresh *H. amplexicaulis* seeds from the north of Australia, Campbell et al. (2009) registered 46% of the seeds as viable, results that are closely similar to 41% seed able to germinate that was found here. Therefore, although in the native area there is no significant growth of *H. amplexicaulis* as macrophyte of the reservoir of the Yacyretá dam, its invasive capacity is similar to that preset in Australia where its colonizing behavior is very aggressive. Germination began earlier than in the experiments conducted by Campbell et al. (2009), who reports that germination began at four and a half days after

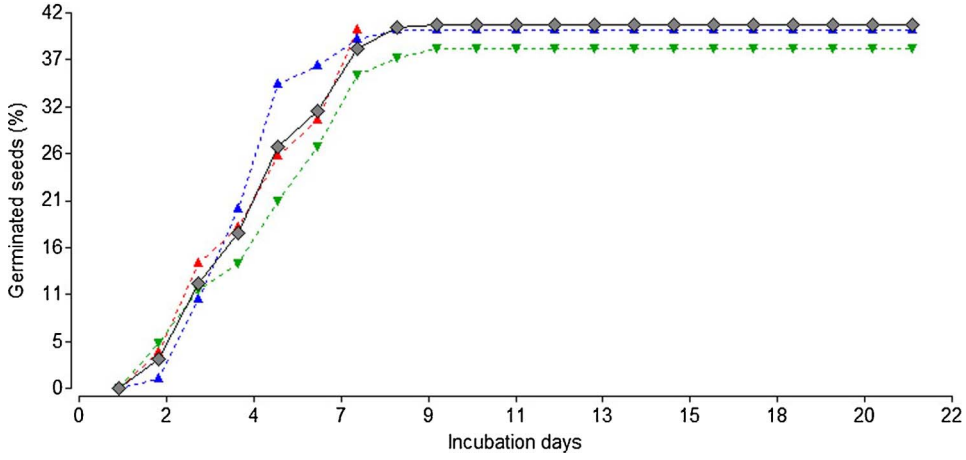

Fig. 2. Germination behaviour through time of seeds of *Hymenachne amplexicaulis*, expressed in accumulative percentage of seed germinated. Note that the end of germination occurs at the 8th day. Color lines represent each essay. Black line represents the means of germination percentage of three replicates.

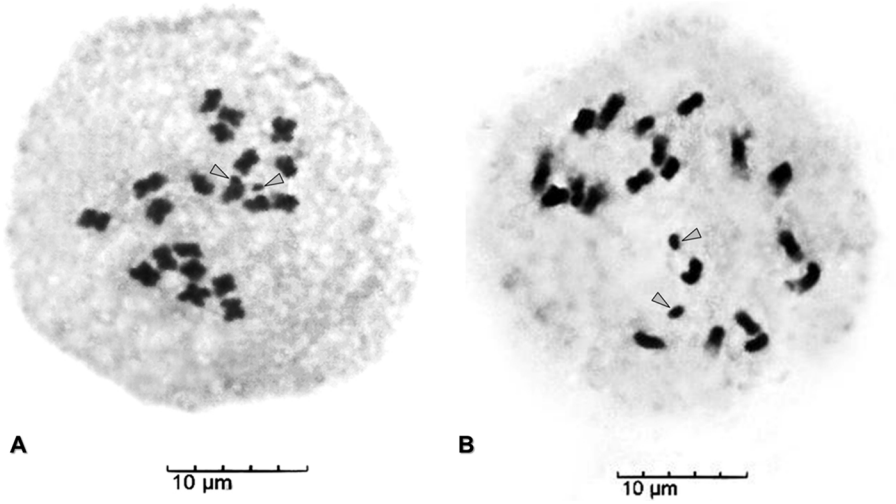

Fig. 3. Mitotic metaphases ($2n = 2x = 20 + 2B$) of *Hymenachne amplexicaulis*. A-B. Notice the smaller size of B chromosomes than the A complement. Arrows show the B chromosomes. Scale bars = 10 µm.

sowing, and reached maximum germinative power at day six. Also, the germination power and germination velocity index for *H. amplexicaulis* in native population was high when compared to the null results of Souza da Silva et al. (2012) when carrying out trials in conditions with alternates temperatures (20 °C/30 °C) and a photoperiod of 12 h (light/dark: 12/12).

Seed viability and seed set production as fertility parameters to estimate reproductive performance of grasses, are widely used to characterize germplasm and also to evaluate weed sexual invasive potential. In a broad context, variations in the seed production of the most diverse nature have been shown in several weed grasses. *Phragmites australis* (Cav.) Trin. ex Steud. (common reed) is one of the most widespread plant species in the world, and present in general, the seed set ability highly heterogeneous, varying by place location of population (McKee and Richards, 1996; Kettenring et al., 2011 and references therein). Flower fertility of *P. australis*, can be in the range < 1% to 5–21% (Kettenring and Whigham, 2009), and also, variations from 0 to 100% and a clear-cut trend of increasing seed set with latitude (McKee and Richards, 1996). It has been suggested several factors to explain low seed set production in *P. australis*, as seasonal rainfalls, temperatures, high polyploid levels, abnormal pollen formation, insect and fungal seed damage, self-sterility, latitude gradient (McKee and Richards, 1996 and references therein), pollen limitation brought about by the clonal structure (Ishii and Kadono, 2002) and depauperate local levels of genetic diversity (Kettenring et al., 2010). Likewise, invasive *Spartina alterniflora* Loisel. seed viability, vary from 4.2% to 35.7% in populations from China (Xiao et al., 2009), and essays carried in *Pennisetum setaceum* (Forssk.) Chiov. (fountain grass), one of the most aggressive of the African grasses in Hawaii, show low field seed-set rates of less than 6%, but greenhouse seed-set rates increased to 10%–15% following resource addition (Goergen and Daehler, 2001). Seed fertility comparisons between weed species or between native versus alien species also show a very heterogeneous conditions, for example, rates of flowering, seed production, and germination were higher in alien invasive than native form of *S. alterniflora* (Callaway and Josselyn, 1992).

The chromosome number found in native individuals of *H.*

Table 2
Quantitative parameters of karyotype of *Hymenachne amplexicaulis*.

Pair	s (µm) ± SE	l (µm) ± SE	c (µm) ± SE	I	r	Type
1	0.97 ± 0.02	1.20 ± 0.04	2.18 ± 0.05	44.67	1.24	m
2	0.96 ± 0.04	1.10 ± 0.04	2.05 ± 0.05	47.00	1.14	m
3	0.90 ± 0.03	1.04 ± 0.03	1.94 ± 0.05	46.24	1.16	m
4	0.83 ± 0.04	1.04 ± 0.03	1.86 ± 0.05	44.36	1.25	m
5	0.79 ± 0.02	0.96 ± 0.03	1.75 ± 0.04	45.22	1.21	m
6	0.80 ± 0.03	0.91 ± 0.03	1.71 ± 0.05	46.67	1.14	m
7	0.74 ± 0.04	0.89 ± 0.02	1.63 ± 0.04	45.53	1.20	m
8	0.67 ± 0.02	0.82 ± 0.03	1.49 ± 0.04	44.67	1.24	m
9	0.65 ± 0.02	0.76 ± 0.03	1.41 ± 0.05	46.02	1.17	m
10	0.57 ± 0.02	0.73 ± 0.03	1.30 ± 0.05	44.08	1.27	m

I = centromeric index; c = chromosome length; l = long arm length; s = short arm length; r = short/long arm ratio. SE = standard error.

Table 3
Karyotype parameters of *Hymenachne amplexicaulis*.

$2n$	20 + 2 B
x	10
Karyotype	20 m + 2 B
TCL	34.65 µm
c	1.73 µm
c max	2.17 µm
c min	1.3 µm
i	45.44
A_1	0.17
A_2	0.16
R	1.68
r > 2	0
Stebbins category	1A

A_1, A_2 = intrachromosomal and interchromosomal asymmetry indices; c = mean chromosome length; c max, c min = maximum and minimum chromosome length; i = mean centromeric index; r > 2 = proportion of chromosome pairs with arm ratio > 2; R = largest/smallest chromosome ratio; TCL = total complement length.

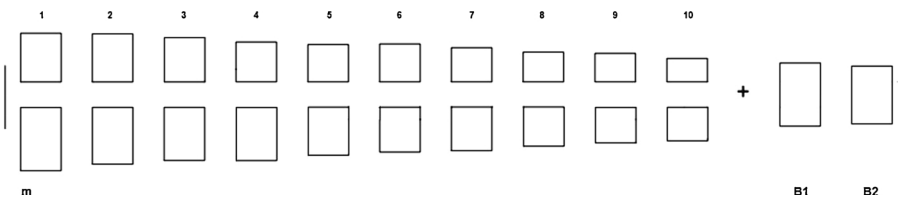

Fig. 4. Representative idiogram of *Hymenachne amplexicaulis*. Karyotype formed by 20 m + 2B. Scale bar = 1 µm.

amplexicaulis differs from those counts previously carried out in America, and this is the first finding of B chromosomes for the genus *Hymenachne*. Previous chromosome counts of $2n = 20$ chromosomes were registered in areas to which the species is native, and the present study is the southernmost chromosome count, seeing as it is the first to be undertaken with specimens from Argentina. According to latest phylogenetic and molecular studies, *Hymenachne* belongs to the Paspaleae tribe, which embrace genera with a basic number of $x = 10$ (Morrone et al., 2012). Previous counts for *Hymenachne* also exist, which differ from $x = 10$, such as $2n = 24$ (Gould and Soderstrom, 1970) in individual plants from Colombia. Other species of the genus have registered chromosomal antecedents of native accessions from America and all agree with $x = 10$, diploids as well as polyploids, like $2n = 40$ for *H. donacifolia* (Honfi et al., 1990) of Costa Rica, Argentina, and Brazil, for *H. pernambucense* (Dubcovsky and Zuloaga, 1991) of Argentina and for *H. grumosa* (Núñez, 1952) from Argentina; and $2n =$ ca.20 for *H. donacifolia* from Bolivia. An exceptional count of $2n = 24$ for *H. donacifolia* was made in Colombia (Gould and Soderstrom, 1970). To the present no polyploidy has been registered for *H. amplexicaulis*, a condition present in the genus (Honfi et al., 1990).

The chromosomal attributes help to understand the variation in fertility of numerous plants, including weeds. For example, *Phragmites australis* present a high phenotypic variation that can be related to variance in chromosome numbers, because a large range in polyploid levels has been found, aneuploids also occur regularly, and cytogeographically, populations in Europe are dominated by tetraploids, whereas octoploids predominate in Asia (Clevering and Lissner, 1999). In the same way, in *Arundo donax* L. a highly polyploid grass species ($18x$) the low seed fertility was considered as a consequence of its ploidy level, however in a recent work, was proposed that polyploidy is not entirely responsible of seed set absence but rather, several early alterations during gametogenesis and pollen germination (Hardion et al., 2015, and references therein). B chromosomes have numerous measurable effects on the nuclear phenotype as well as on the plant as a whole, and in general, as they increase in number their effects are deletereous, diminishing the fertility of the carrier (Jones, 1995). Generally, the fertility of the carrier is considered very sensitive to the presence of supernumerary chromosomes, and detrimentally so (Jones et al., 2008), such that diminution in fertility tends to be associated with the presence of B chromosomes (Jones and Houben, 2003). In *H. amplexicaulis*, the male fertility evaluated through pollen resulted high, suggesting that the paternal contribution is not affected by the presence of B chromosomes or others factors. On the other hand, the effects of B chromosomes on other seminal parameters and on germination are wide ranging, depending on the species in question. Moss (1966) observed that B chromosomes increase the variance in the weight of the seeds, germination times, and fertility in rye (*S. cereale*). Puertas et al. (1985) calculated various components of reproductive efficiency for *Secale vavilovii* Grossh. and *S. cereale* which had from 0 to 4 B chromosomes and a positive correlation was found between a decrease in fertility and an increase in the number of B chromosomes, with similar results in both species. In native *H. amplex*icaulis carrying B chromosomes, the production of the seed set is not high but viable seeds have a very good germinative capacity, similar than areas where is invasive. Further detailed studies, like meiotic behaviour analyses and cytogeography, can help to understand the role of B chromosomes in populations dynamic and its relationships with potential fertility. Native populations of *H. amplexicaulis* situated over rivers (i.e. Paraná River) and streams (i.e. Yabebiry stream) are subject to flooding pulses and other hydrological dynamics, and thus are conditioned to fluctuations in the currents and water levels, as well as to the derived effects on the riverbank or coastal areas. The establishment and permanence in these environments, where *H. amplexicaulis* is considered one of the typical native macrophytes of the region, has a direct link to the reproductive performance of its populations. Further studies should focusing to test if the presence of B chromosomes can explain the non-invasive behaviour by sexual means of native populations of *H. amplexicaulis*, and the reproductive dynamic of B host plants, throughout the entire reproductive cycle, and during demographic oscillations. Besides, the vegetative reproduction can also offer a solution to maintain the B chromosomes in the population while avoiding its extinction.

5. Conclusion

Characterizing the fertility for invasive riparian species could assist with detecting mechanisms of dispersal and enable predictions of invasiveness. Management regimes should target elimination and limit widespread of this macrophyte particularly, because rivers and streams serve as an effective dispersal corridor for macrophytes range expansion. Currently, in spite of the reproductive performance, we suspect that the invasive potential by asexual means is functional, but is not expressed because Parana River and tributaries streams are subjected to pulse dynamics variation of water levels.

In *H. amplexicaulis* effective fertility is lower than its potential fertility, but it is not an indicator of absence of invasive threat. Based on plant seed fertility, the potential risk of invasiveness seems to be limited, however the reproductive performance of B host plants indicates sufficient invasive potential by sexual means. In the future, studies of cytogenetics and ecological dynamics of natural populations, and comparisons between sexual and asexual reproductive performance will shed light for a proper management of control policies to prevent invasions of this species.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

Araya, P., Hirt, L., Flores, S., et al., 2013. Humedales de los arroyos de Misiones en relieve escarpado. In: Benzaquén, L. (Ed.), Inventario de los Humedales de Argentina: Sistemas de paisajes de humedales del Corredor Fluvial Paraná-Paraguay, 1st ed. Secretaría de Ambiente y Desarrollo Sustentable de la Nación, Buenos Aires, pp. 137–146.

Baker, H.G., 1965. Characteristics and modes of origin of weeds. In: Baker, Stebbins (Eds.), The Genetics of Colonizing Species. Academic Press, New York, pp. 147–168.

Bosemark, N.O., 1957. On accesory chromosomes in *Festuca pratensis* V: influence of accessory chromosomes on fertility and vegetative development. Hereditas 43, 211–235.

Brasil, 2009. Regras para análise de sementes. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defensa Agropecuária, Brasília, pp. 1–399.

Braza, R., Arroyo, J., García, M.B., 2010. Natural variation of fecundity components in a widespread plant with dimorphic seeds. Acta Ecol. 36, 471–476.

Burns, J.H., Pardini, E.A., Schutzenhofer, M.R., Chung, Y.A., Seidler, K.J., Knight, T.M., 2013. Greater sexual reproduction contributes to differences in demography of invasive plants and their noninvasive relatives. Ecology 94 (5), 995–1004.

Callaway, J.C., Josselyn, M.N., 1992. The introduction and spread of smooth cordgrass *(Spartina alterniflora)* in South San Francisco Bay. Estuaries 15 (2), 218–226.

Camacho, J.P.M., Sharbel, T.F., Beukeboom, L.W., 2000. B-chromosome evolution. Philos. Trans. R. Soc. B –Biol. Sci. 355 (1394), 163–178.

Campbell, S.D., Carter, E.A., Setter, M.J., 2009. Germination of *Hymenachne amplexicaulis* and *H acutigluma* under contrasting light, temperature and nitrate regimes. Plant Prot. Q. 24 (1), 10–14.

Clevering, O.A., Lissner, J., 1999. Taxonomy chromosome numbers, clonal diversity and

population dynamics of *Phragmites australis*. Aquat. Bot. 64, 185–208.
Csurhes, S.M., Mackey, A.P., Fitzsimmons, L., 1999. Hymenachne (*Hymenachne amplexicaulis*) in Queensland. Pest Status Review Series – Land Protection. Department of Natural Resources and Mines, Queensland, pp. 1–39.
Dubcovsky, J., Zuloaga, F.O., 1991. Números cromosómicos de especies sudamericanas de *Panicum* (Poaceae: Paniceae). Bol. Soc. Argent. Bot. 27, 201–206.
Enriquez-Quiroz, J.F., Quero-Carrillo, A.R., Hernandez-Garay, A., Garcia-Moya, E., 2006. Azuche, *Hymenachne amplexicaulis* (Rudge) Nees, forage genetic resources for floodplains in tropical Mexico. Genet. Resour. Crop Evol. 53, 1405–1412.
Goergen, E., Daehler, C.C., 2001. Reproductive ecology of a native Hawaiian grass (*Heteropogon contortus*; Poaceae) versus its invasive alien competitor (*Pennisetum setaceum*; Poaceae). Int. J. Plant Sci. 162 (2), 317–326.
González-Sánchez, M., Chiavarino, M., Jiménez, G., Manzanero, S., Rosato, M., Puertas, M.J., 2004. The parasitic effects of rye B chromosomes might be beneficial in the long term. Cytogenet. Genome Res. 106, 386–393.
Gould, F.W., Shaw, R.B., 1992. Gramíneas. Clasificación Sistemática. AGT Editor, S.A, México.
Gould, F., Soderstrom, T., 1970. Chromosome numbers of some Mexican and Colombian grasses. Can. J. Bot. 48, 1633–1639.
Grant, V., 1989. Especiación vegetal. Primera edición. Editorial Limusa S. A., México.
Hacker, J.B., Loch, D.S., 1997. Tropical forage seed production: producers' views and research opportunities. In: Proceedings of the XVIII International Grassland Congress. Winnipeg.
Hardion, L., Verlaque, R., Rosato, M., Roselló, J.A., Vila, B., 2015. Impact of polyploidy on fertility variation of mediterranean Arundo L. (Poaceae). C. R. Biol. 338, 298–306.
Honfi, A.I., Quarin, C.L., Valls, J.F.M., 1990. Estudios cariológicos en gramíneas sudamericanas. Darwiniana 30, 87–94.
Hussner, A., Stiers, I., Verhofstad, M.J.J.M., Bakker, E.S., Grutters, B.M.C., Haury, J., van Valkenburg, J.L.C.H., Brundu, G., Newman, J., Clayton, J.S., Anderson, L.W.J., Hofstra, D., 2017. Management and control methods of invasive alien freshwater aquatic plants: a review. Aquat. Bot. 136, 112–137.
Ishii, J., Kadono, Y., 2002. Factors influencing seed production of *Phragmites australis*. Aquat. Bot. 72, 129–141.
Jones, N., Houben, A., 2003. B chromosomes in plants: escapees from the A chromosome genome? Trends Plant. Sci. 8 (9), 417–423.
Jones, R.N., Viegas, W., Houben, A., 2008. A century of B chromosomes in plants: so what? Ann. Bot 101, 767–775.
Jones, R.N., 1995. B chromosomes in plants. tansley review No. 85. New Phytol. 131, 411–434.
Kettenring, K.M., Whigham, D.F., 2009. Seed viability and seed dormancy of non-native Phragmites australis in suburbanized and forested watersheds of the Chesapeake Bay, USA. Aquat. Bot. 91, 199–204.
Kettenring, K.M., McCormick, M.K., Baron, H.M., Whigham, D.F., 2010. *Phragmites australis* (common reed) invasion in the Rhode River subestuary of the Chesapeake Bay: disentangling the effects of foliar nutrients, genetic diversity, patch size, and seed viability. Estuaries Coasts 33, 118–126.
Kettenring, K.M., McCormick, M.K., Baron, H.M., Whigham, D.F., 2011. Mechanisms of Phragmites australis invasion: feedbacks among genetic diversity, nutrients, and sexual reproduction. J. Appl. Ecol. 48, 1305–1313.
Levan, A., Fredga, K., Sandberg, A.A., 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52, 201–220.
Lloyd, D.G., 1979. Parental strategies of angiosperms. N. Z. J. Bot. 17, 595–606.
Lloyd, D.G., 1980. Sexual strategies in plants I. An hypothesis of serial adjustments of maternal investment during one reproductive session. New Phytol. 86, 69–79.
Maguire, J.D., 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 2, 176–177.
McKee, J., Richards, A.J., 1996. Variation in seed production and germinability in common reed *(Phragmites australis)* in Britain and France with respect to climate. New Phytol. 133, 233–243.
Morrone, O., Aagesen, L., Scataglini, M.A., Salariato, D.L., Denham, S.S., Chemisquy, M.A., Sede, S.M., Giussani, L.M., Kellogg, E.A., Zuloaga, F.O., 2012. Phylogeny of the Paniceae (Poaceae: panicoideae): integrating plastid DNA sequences and morphology into a new classification. Cladistics 28, 333–356.
Moss P., 1966, The adaptive significance of B-chromosomes in rye, C.D. Darlington, K.R. Lewis (Eds), Chromosomes Today, vol. I, Edinburgh, 15–23.
Müntzing, A., 1943. Genetical effects of duplicated fragment chromosomes in rye. Hereditas 29, 234–282.
Núñez, O., 1952. Investigaciones Cariosistemáticas en las Gramíneas Argentinas de la tribu Paniceae. Rev. Fac. Agron. La Plata 28 (2), 229–255.
Nicora, E., Rúgolo De Agrassar, Z., 1987. Los géneros de Gramíneas de América Austral (Argentina, Chile, Uruguay y áreas limítrofes de Bolivia, Paraguay y Brasil). Hemisferio Sur, Buenos Aires, Argentina.
Puertas, M.J., Romera, F., Delapena, A., 1985. Comparison of B chromosome effects on *Secale cereale* and *Secale vavilovii*. Heredity 55 (2), 229–234.
Randolph, L.R., 1941. Genetic characteristics of the B- chromosome in maize. Genetics 26, 608–631.
Romero Zarco, C., 1986. A new method for estimating karyotype asymmetry. Taxon 3, 531–536.
Sellers, B.A., Diaz, R., Overholt, W.A., Langeland, K.A., Gray, C.J., 2008. Control of West Indian marsh grass with glyphosate and imazapyr. J. Aquat. Plant. Manag. 46, 189–192.
Souza da Silva, K., de Oliveira Machado, S.L., de Menezes, N.L., Kurtz Urban, L.J., Palma Alves, M.V., 2012. Methodology adequation of tetrazolium test for Hymenachne amplexicaulis seeds. Semina: ciências Agrárias. Londrina 33 (5), 1819–1824.
Stebbins, G.L., 1971. Chromosomal Evolution in Higher Plants. Addison- Wesley Co., New York.
Stephenson, A.G., 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annu. Rev. Ecol. Evol. Syst. 12, 253–279.
Wearne, L.J., Clarkson, J., Grice, A.C., Van Klinken, R.D., Vitelli, J.S., 2010. The biology of australian weed 56. *Hymenachne amplexicaulis* (Rudge) nees. Plant. Prot. Q. 25 (4), 146–161.
Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A., Wright, I.J., 2002. Plant ecological strategies: some leading dimensions of variation between species. Annu. Rev. Ecol. Syst. 33, 125–159.
Xiao, D., Zhang, L., Zhu, Z., 2009. A study on seed characteristics and seed bank of Spartina alterniflora at saltmarshes in the Yangtze Estuary. China Estuar. Coast. Shelf Sci. 83, 105–110.
Zuloaga, F.O., Morrone, O., Rúgolo De Agrasar, Z.E., Anton, A.M., Arriaga, M.O., Cialdella, A.M., 1994. Gramineae V. Panicoideae, Paniceae, *Acroceras* Stapf–*Panicum* L. Flora Del Paraguay. 23: I-IV. Conservatoire Et Jardin Botaniques De La Ville De Geneve. Missouri Bot. Gard, Geneva, St. Louis.
Zuloaga, F.O., Morrone, O., Davidse, G., Filgueiras, T.S., Peterson, P.M., Soreng, R.J., Judziewicz, E., 2003. Catalogue of new world grasses (Poaceae): III. Subfamilies Panicoideae, Aristidoideae, Arundinoideae and Danthonioideae. Contrib. U. S. Nat. Herb. 46.