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Environmental influence on reproductive traits of buffel grass facultative apomictic genotypes and its implication in plant breeding

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Abstract

Cenchrus ciliaris L., a perennial C4 grass, is one of the most widely used forage species in warm and dry regions worldwide. The widespread use of the cultivar Texas raises concerns about genetic homogeneity and vulnerability. Hybridizations among facultative individuals could widen the genic pool. Therefore, a reproductive characterization of available genotypes is required. The objective of this study was to determine the reproductive mode, potential sexual expressivity, and seed fertility of four facultative apomictic genotypes throughout the flowering season, considering the influence of bioclimatic variables. We assessed the reproductive mode and potential sexual expressivity using the pistil clearing technique and seed fertility based on the production of seeds per panicle under open pollination and self-pollination. All the assessed genotypes behaved mainly as out-crossers throughout the flowering season and showed the highest potential sexual expressivity at the beginning of flowering. We confirmed the environmental influence on reproductive traits. At the beginning of the flowering season, the facultative apomictic genotypes exhibited the highest potential as female parent for hybridizations programs.

1 | INTRODUCTION

Cenchrus ciliaris L. (buffel grass) is a warm-season perennial C4 grass (Hanselka et al., 2004) that has proved useful in a wide range of environments due to its high biomass yield, drought tolerance, resistance to overgrazing, high efficiency in the use of nutrient and water resources, and capacity for

carbon sequestration and soil erosion control (Al-Dakheel & Hussain, 2016). It is an important gene source for abiotic stress tolerance in the current climate change scenario, especially in arid and semiarid regions worldwide. Buffel grass is one of the most widely used forage species (Kharrat Souissi et al., 2013; Negawo et al., 2020). However, few cultivars are known, with Texas being the most widely used (Gómez Martínez, 2009; Griffa et al., 2017); this fact raises concerns about genetic vulnerability to changing environmental conditions (Parker et al., 2022).

Aposporous apomixis is the main reproductive mode in buffel grass (Negawo et al., 2020; Quiroga et al., 2013; Sharma et al., 2014), followed by pseudogamy (Shafer et al., 2000), with fully sexual sources being absent or very rare (Bashaw, 1962; Kumar et al., 2010). The absence of completely sexual plants has greatly restricted gene transfer through male gametes. Thus, the breeding of these species is limited to ecotype selection due to the difficulty of recombining genes through controlled crosses. Facultative apomicts were reported in this species (Hignight et al., 1991; Quiroga et al., 2013; Sharma et al., 2014). These plants can bear meiotic and aposporic embryo sacs in the same plant or even within the same ovule. The coexistence of meiotic and aposporic embryo sacs within the same ovule (potential sexual expressivity), as reported in other species (Hojsgaard et al., 2013; Rebozzio et al., 2011; Reutemann et al., 2022; Zilli, 2016), in addition to the low effective sexual pathway (Hussey et al., 1991), provides buffel grass potential for hybridization to obtain segregating offspring (Hörandl & Hojsgaard, 2012). Therefore, although most of the progeny are formed asexually and have a maternal genotype, the capacity to form seeds via sexual reproduction is also retained. Consequently, as reported by Novo et al. (2020), controlled crosses between facultative apomicts and obligate apomicts (used as female and male parents, respectively) could allow new and desirable genetic combinations. For hybridization purposes, facultative apomictic genetic resources should gather desirable agronomic traits, high sexual expression, and cross-fertility (Acuña et al., 2007).

Apomixis inheritance has been frequently studied in buffel grass (Akiyama et al., 2005; Conner et al., 2013; Ozias-Akins et al., 2003; Rathore et al., 2020, 2022; Syamaladevi et al., 2016). The epigenetic control of apomixis expression is an emerging theory that suggests that apomixis is reversibly superimposed upon sexuality (Kumar, 2017). Although there is evidence of epigenetic control of the reproductive mode in several *Poaceae* species (Karunarathne et al., 2020; Reutemann et al., 2022; Rios et al., 2013; Soliman et al., 2021), the environmental influence on apospory/sexual expression throughout the flowering season has been scarcely explored in buffel grass (Gounaris et al., 1991; Hussey et al., 1991) under field conditions.

Core Ideas

- Reproductive behavior of *C. ciliaris* is influenced by bioclimatic variables throughout the flowering season.
- The highest potential sexual expressivity occurs at the beginning of flowering, with the longest photoperiod.
- Precipitation, thermal time, and photoperiod are the main bioclimatic variables explaining seed fertility variation.
- The lowest self-compatibility occurs at the beginning and peak of flowering.

Buffel grass is a protogynous (Shafer et al., 2000) and mainly cross-pollinated (Hanselka et al., 2004) species with variable levels of self-compatibility. On the other hand, self-incompatibility promotes cross-pollination, preventing inbreeding depression; this evolutionary strategy is widespread among perennial grasses (Do Canto et al., 2016) such as buffel grass. Even though some studies in other species have reported the environmental influence on self-incompatibility, measured as seed fertility (Gutterman, 2000; Kissling et al., 2006; Krishnan & Rao, 2005; Zilli et al., 2018), there are no reports of self-incompatibility variation during the flowering season in buffel grass under field conditions (Conde-Lozano et al., 2011; Gómez Martínez, 2009).

The objective of this study was to determine the reproductive mode, potential sexual expressivity, and seed fertility of four buffel grass facultative apomictic genotypes throughout the flowering season, considering the influence of bioclimatic variables.

2 | MATERIALS AND METHODS

The study involved four highly apomictic genotypes exhibiting residual sexuality: a progeny of B-1s (Sx) (kindly provided by the University of Texas); two F1 hybrids, named 32/9 and 191, obtained by crossing Sx with two different apomictic cultivars (cv. Molopo and cv. Messina, respectively) (Griffa, 2010; Quiroga et al., 2013); and an S1 plant originated from self-pollination of Sx genotype. The study was conducted in two growing seasons (2018/2019 and 2019/2020). All genotypes were agamically multiplied in autumn 2018, and three vegetative clones per genotype were planted in late spring (November 2018) in a plot isolated from other buffel grass pollen sources (31.47° S, 64.15° W, Córdoba, Argentina), under a nylon cover structure, with 1-m spacing within and between rows, in a completely randomized

design. Two spring mowings were performed to homogenize all clones: one of them was practiced right after planting (second half of November 2018) and the other at the beginning of the following growing season (second half of November 2019).

In each of the two growing seasons, we defined three flowering periods according to the percentage of panicles at anthesis from each clonal plant per genotype (experimental unit)—beginning of flowering (BF, 25%) corresponding to late spring, peak of flowering (PF, 50%) corresponding to mid-summer, and end of flowering (EF, 75%) corresponding to early fall—to evaluate target reproductive traits: reproductive mode, potential sexual expressivity, and seed fertility. More than 500 pistils per genotype were observed and classified to determine the reproductive mode and potential sexual expressivity. Moreover, to determine seed fertility, more than 50 panicles per genotype were evaluated.

2.1 | Reproductive mode and potential sexual expressivity

In each flowering period (BF, PF, and EF) of each of the two growing seasons, three inflorescences at the protogyny stage (one panicle from each of the three clonal plants) were randomly selected from each genotype and fixed in a 70% ethanol, 37% formaldehyde, and glacial acetic acid (18:1:1) solution for 24–48 h. Panicles were then placed in vials containing 70% ethanol solution.

Central spikelet pistils from the middle part of each inflorescence were dissected and cleared using the procedure described by Young et al. (1979), with modifications by Sharma et al. (2014), Zilli et al. (2015), and our own, as follows: they were placed in 2-mL tubes, dehydrated by passing them through an ethanol series for 30 min each (1 × 85% ethanol, 2 × 100% ethanol), and kept in 100% ethanol overnight. The following day, they were passed through an ethanol:methyl salicylate series for 30 min each (ethanol:methyl salicylate, 50:50 v/v; ethanol:methyl salicylate, 25:75 v/v; ethanol:methyl salicylate, 15:85 v/v and 100 methyl salicylate) and finally kept in 100% methyl salicylate for a month. The solutions were replaced with a Pasteur pipette. More than 500 pistils per genotype were mounted in 100% methyl salicylate and observed under bright field microscopy using DIC mode at 20×-40× (Nikon Eclipse TI confocal microscope).

Embryo sacs were classified according to their morphology (Young et al., 1979; Zilli, 2016) as *Polygonum* or *Panicum* type. Megagametophytes containing an egg cell, a mono- or bi-nucleated central cell, and a chalazal mass of antipodal cells (*Polygonum* type) were classified as meiotic embryo sacs (MES) (Figure 1a). Those bearing an egg cell, a central cell with one or two nuclei, and no antipodals (*Panicum* type) were classified as aposporic embryo sacs (AES)

(Figure 1b). Besides, when one or more aposporic embryo sacs and one or two meiotic embryo sacs were observed, they were classified as multiple mixed embryo sacs (MMES) (Figure 1c), and when more than one aposporic embryo sacs were observed, they were considered multiple aposporic embryo sacs (MAES) (Figure 1d). Immature and aborted embryo sacs (Zilli, 2016) were also recorded; however, since it was not possible to categorize them as either sexual or aposporic, they were discarded.

To estimate the reproductive mode, the total number of MES was divided by the total number of observed embryo sacs (TES) (Equation 1). Moreover, according to the method of Zilli (2016), potential sexual expressivity (PSE) was calculated by summing MES plus MMES and dividing by TES (Equation 2). The values were expressed as proportion or as percentage, depending on the statistical analysis performed.

$$\text{Reproductive mode} = \frac{\text{MES}}{\text{MES} + \text{AES} + \text{MMES} + \text{MAES}} = \frac{\text{MES}}{\text{TES}}, \quad (1)$$

$$\text{PSE} = \frac{\text{MES} + \text{MMES}}{\text{TES}}. \quad (2)$$

2.2 | Seed fertility

Self-incompatibility was estimated as seed fertility based on procedures described by Nakagawa et al. (1987) and Zilli et al. (2018). Seed fertility was determined as the number of caryopses per spikelet per panicle (seed set) under two treatments, open pollination (OP) and self-pollination (SP), in three moments of the flowering period (BF, PF, and EF) in two growing seasons (2018/2019 and 2019/2020).

To obtain SP seed set, in each flowering period during each growing season, three to seven panicles per genotype (one to three panicles from each clonal plant per genotype) were enclosed in acetate tubes prior to stigma exertion until harvest. Open-pollinated seed set, in each flowering period during each of the two growing seasons, was obtained by labeling three to seven immature panicles (i.e., panicles without exerted reproductive organs) per genotype (one to three panicles from each clonal plant per genotype), which were exposed to natural pollination. Emasculation was not needed because buffel grass is a protogynous species (Shafer et al., 2000).

Nearly one month after the beginning of each pollination treatment, OP and SP panicles were individually harvested and threshed. For each panicle, the number of spikelets and caryopses was determined. Seed set was calculated as the quotient between number of caryopses and number of spikelets per panicle of each clonal plant per genotype and was expressed as proportion or as percentage, depending on the statistical analysis performed.
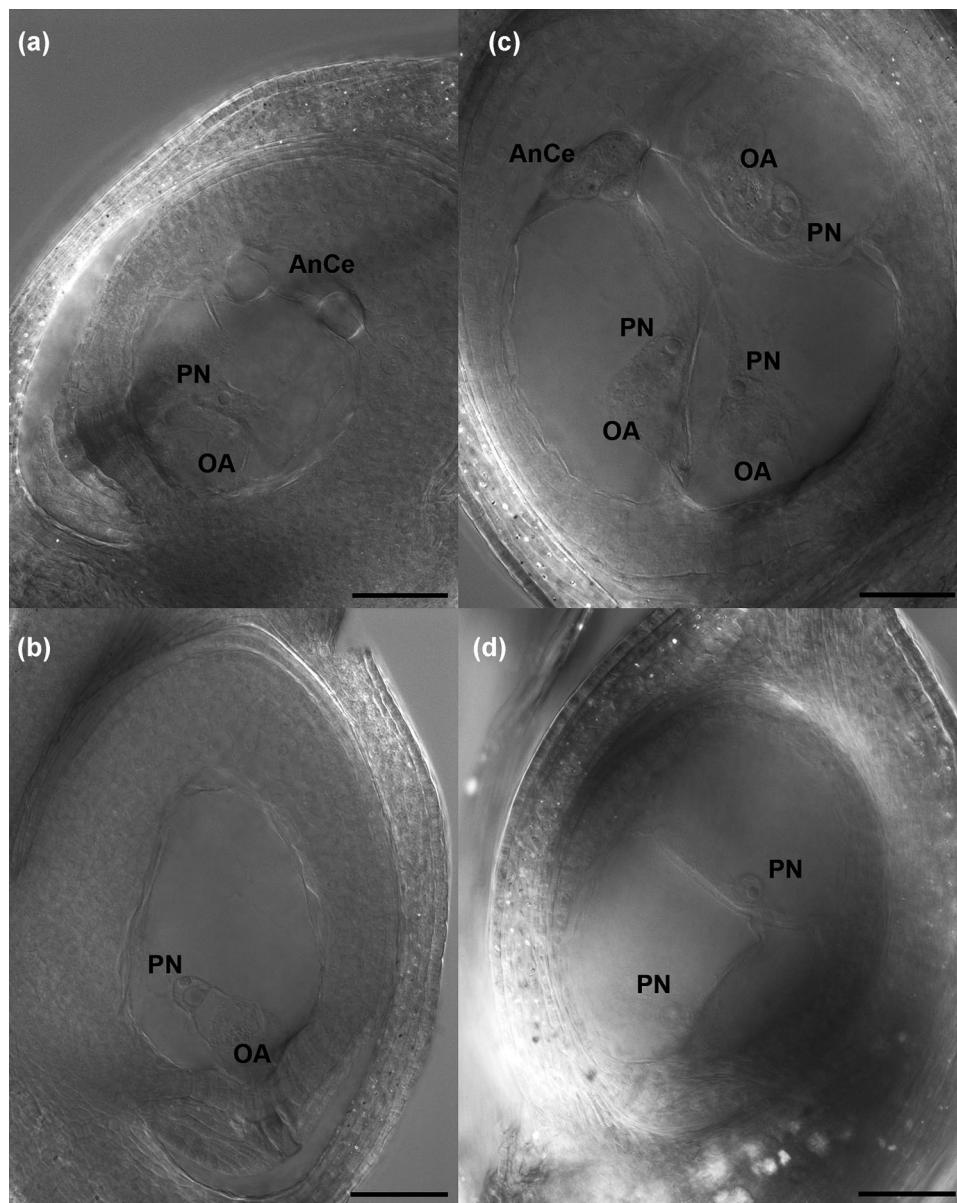
FIGURE 1 *Cenchrus ciliaris* L. (buffel grass) embryo sac types (scale bar = 100 μm): (a) meiotic embryo sac (MES), (b) aposporic embryo sac (AES), (c) multiple mixed embryo sac (MMES), and (d) multiple aposporic embryo sac (MAES). AnCe, antipodal cells; OA, oospheric apparatus; PN, polar nucleus/nuclei.

2.3 | Bioclimatic variables

To assess the environmental influence on the evaluated traits, thermal time (°C day^{-1}) calculated considering 12°C as buffel grass base temperature (Ávila et al., 2014), cumulative precipitation (mm), average photoperiod length (h day^{-1}), and cumulative radiation (W (m^2)$^{-1}$) values were recorded in the same flowering periods (beginning, peak, and end of flowering) throughout the two growing seasons, but at different times ranges within those periods for the different assessed traits. Average values were then calculated. All bioclimatic values were obtained from the Laboratorio de Hidráulica from Facultad de Ciencias Exactas, Físicas y Naturales-Universidad Nacional de Córdoba weather station web system (Omixom, 2022). For the reproductive mode and PSE, bioclimatic variables were recorded from spring mowing (second half of November) to each panicle sampling date in each flowering period (Table 1).

Regarding seed fertility, bioclimatic variables were recorded from 10 days before the beginning of each pollination treatment (OP or SP) until harvest for each panicle of each clonal plant per genotype (Table 2) in each flowering period (BF, PF and, EF).

TABLE 1 Influence of bioclimatic variables on reproductive mode and potential sexual expressivity (PSE) in buffel grass genotypes: mean values of bioclimatic variables and corresponding coefficients of variation (CV) prevailing from spring mowing to panicle sampling date in each flowering period (BF: beginning of flowering, PF: peak of flowering, and EF: end of flowering).

Flowering period	Thermal time (°C day^{-1})		Cumulative radiation (W (m^2)$^{-1}$)		Average photoperiod length (h day^{-1})		Cumulative precipitation (mm)	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
BF	444.4	31.76	323.07	5.12	14.54	0.56	166.83	8.02
PF	1041.72	16.68	307.72	4.88	14.09	0.77	340.33	13.8
EF	1536.98	15.13	299.26	1.7	13.44	2.28	419.84	20.93

TABLE 2 Influence of bioclimatic variables on seed fertility in buffel grass genotypes: mean values of bioclimatic variables and corresponding coefficients of variation (CV) recorded from 10 days before the beginning of each pollination treatment to harvest in each flowering period (BF: beginning of flowering, PF: peak of flowering, and EF: end of flowering).

Flowering period	Thermal time (°C day^{-1})		Cumulative radiation (W (m^2)$^{-1}$)		Average photoperiod length (h day^{-1})		Cumulative precipitation (mm)	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
BF	613.78	17.62	13,772.93	19.54	14.25	1.36	154.25	37.21
PF	631.51	21.99	18,435.59	38.18	12.88	4.38	143.06	56.43
EF	399.44	27.6	12,504.46	20.82	11.06	5.4	82.68	97.00

2.4 | Data analyses

To assess the reproductive mode and PSE, a mixed generalized linear model (MGLM) was fitted with binomial distribution and logit link function; the number of embryo sacs of each type (MES or MES + MMES) divided by TES (proportion) was considered the response variable. Genotype effect, flowering period effect, and their interaction were considered fixed effects; bioclimatic variables were considered covariates; and inter-annual variation and correlation among panicles of the same clonal plant per genotype were considered nested random effects. The DGC a posteriori test (p-value ≤ 0.05) (Di Rienzo et al., 2002) was used to compare differences among mean levels of fixed effects. Moreover, PSE (expressed as percentage) was subjected to a partial least squares (PLS) regression analysis. This multivariate statistical method shows the relationship between X data (bioclimatic variables) and Y data (PSE). This data reduction technique reduces the X variables to a set of non-correlated factors that describe the variation in the Y data. In this case, it allows us to explain the common covariance structure and determine the existence of statistically significant cause–effect correlations with bioclimatic variables (predictor variables) for each genotype–flowering period combination, and to identify the bioclimatic variables with the highest incidence.

In addition, an MGLM was fitted with binomial distribution and logit link function for seed fertility considering the proportion between the number of caryopses and the number of spikelets per panicle of each clonal plant per genotype as response variable. Genotype, pollination method, flowering period, and all their two- and three-factor interactions were considered fixed effects, and bioclimatic variables were regarded as covariates. Inter-annual variation and correlation among panicles of the same clonal plant per genotype under each pollination method were considered nested random effects. The DGC a posteriori test (Di Rienzo et al., 2002) (p-value ≤ 0.05) was conducted to compare differences among mean levels of fixed effects. Furthermore, a PLS regression analysis was performed between bioclimatic variables (predictor variables) and seed fertility percentage (response variables) for each genotype-pollination method–flowering period combination. All the statistical analyses were performed using InfoStat Software and its R interface (Di Rienzo et al., 2022; R Core Team, 2021).

3 | RESULTS

No significant differences in the reproductive mode (data not shown) were found among genotypes, flowering period, and genotype–flowering period interaction for all the bioclimatic variables considered. However, a decreasing trend in sexual expression toward EF was noticed, that is, all the genotypes exhibited the greatest amount of sexually derived embryo sacs at BF. On the contrary, there was a statistically significant effect of genotype–flowering period interaction for PSE (Table 3), with greater differences among flowering periods than among genotypes. All the genotypes showed the

T A B L E 3 Potential sexual expressivity (PSE) in buffel grass genotypes (Sx, S1, 32/9, and 191) in three flowering periods (BF: beginning of flowering, PF: peak of flowering, and EF: end of flowering) estimated as meiotic embryo sacs (MES) along with multiple mixed embryo sacs (MMES) divided by the total number of observed embryo sacs (TES) per panicle. Adjusted proportion value ± standard error.

	Flowering period		
Genotype	BF	PF	EF
Sx	0.034 ± 0.011A	0.012 ± 0.002B	0.010 ± 0.003B
S1	0.046 ± 0.014A	0.010 ± 0.002B	0.008 ± 0.002B
32/9	0.041 ± 0.013A	0.010 ± 0.002B	0.010 ± 0.003B
191	0.035 ± 0.010A	0.012 ± 0.002B	0.009 ± 0.003B

Note: Different letters represent significant differences at the $p < 0.05$ probability level.
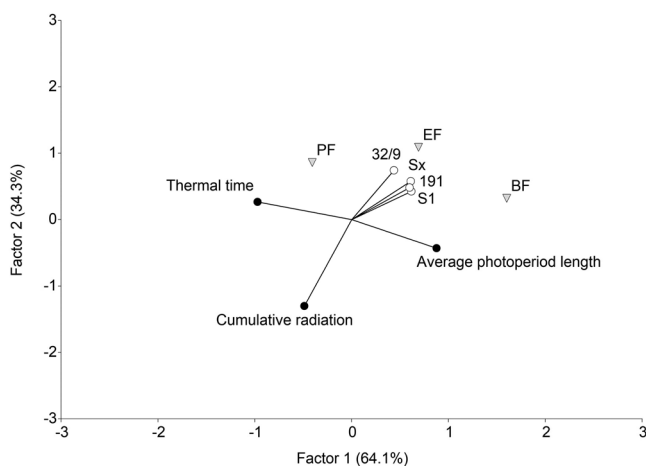

F I G U R E 2 Triplot resulting from partial least squares (PLS) regression analysis between potential sexual expressivity (PSE) and bioclimatic variables in buffel grass genotypes (Sx, S1, 32/9, and 191) in three flowering periods (BF: beginning of flowering, PF: peak of flowering, and EF: end of flowering).

highest PSE at BF (Table 3). Regarding environmental conditions, significant differences were also recorded for all the bioclimatic variables, except for cumulative precipitation.

The factor axes 1 and 2 (Figure 2) of the triplot obtained by PLS regression analysis explained 98.4% of the common covariance structure between PSE and bioclimatic variables in the three flowering periods, except for cumulative precipitation, which was excluded due to its nonsignificant incidence. This statistical approach allowed us to confirm that average photoperiod length was the variable with the highest incidence on PSE (Figure 2), mostly at BF (mean value 14.53 h day^{-1}), and at EF to a lesser degree. Thermal time and cumulative radiation were associated with PF and showed a negative effect on PSE.

The MGLM fitted to seed fertility showed significant differences for the genotype-pollination method–flowering
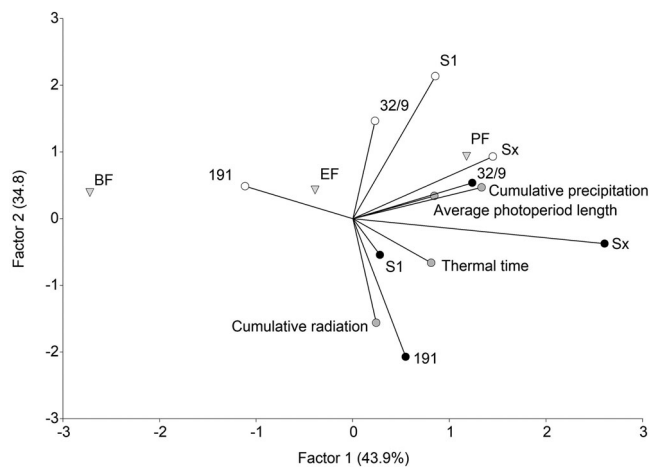

F I G U R E 3 Triplot resulting from partial least squares (PLS) regression analysis between seed fertility and bioclimatic variables (gray dots) in buffel grass genotypes (Sx, S1, 32/9, and 191) under two pollination methods (OP: open pollination [black dots] and SP: self-pollination [white dots]) in three flowering periods (BF: beginning of flowering, PF: peak of flowering, and EF: end of flowering).

period interaction (Table 4). All of the bioclimatic variables were also significant. Seed fertility values of all the genotypes under OP in all flowering periods were above 0.50, except for genotype S1 at BF and EF (0.38 and 0.40, respectively); OP seed fertility values were also higher than all the values corresponding to SP seed fertility, which were below 0.30.

The factor axes 1 and 2 (Figure 3) of the triplot obtained by the PLS regression analysis explained 78.7% of the common covariance structure between seed fertility and bioclimatic variables in the three flowering periods. Cumulative precipitation, thermal time, and average photoperiod length were the main covariates that helped to explain seed fertility variation among genotype-pollination method combinations in each flowering period.

Factor 1 axis showed that cumulative precipitations had the greatest influence on Sx-OP (black dot) seed fertility. On the contrary, the 191-SP (white dot) combination showed to be the most self-compatible at BF. Factor 2 axis allowed us to differentiate and cluster genotypes according to their pollination method (OP or SP). Seed fertility of genotypes 191 and S1, both under OP at PF, was influenced by thermal time and cumulative radiation.

4 | DISCUSSION

In buffel grass, aposporous apomixis is genetically controlled by a linkage block, the apospory-specific genomic region (ASGR) (Akiyama et al., 2004, 2005; Conner et al., 2008; Goel et al., 2003; Ozias-Akins et al., 1998, 2003). As grass genomes are largely collinear and apomixis is spread across

TABLE 4 Seed fertility in four buffel grass genotypes (Sx, S1, 32/9, and 191) in three flowering periods (BF: beginning of flowering, PF: peak of flowering, EF: end of flowering) estimated as the number of caryopses divided by the number of spikelets per panicle. Adjusted proportion value ± standard error.

Genotype	Open pollination			Self-pollination		
	BF	PF	EF	BF	PF	EF
Sx	0.84 ± 0.04A	0.86 ± 0.03A	0.66 ± 0.06B	0.18 ± 0.04D	0.27 ± 0.05C	0.07 ± 0.02E
S1	0.38 ± 0.06C	0.60 ± 0.05B	0.40 ± 0.06C	0.12 ± 0.03D	0.15 ± 0.03D	0.14 ± 0.03D
32/9	0.81 ± 0.04A	0.75 ± 0.04B	0.74 ± 0.05B	0.27 ± 0.05C	0.19 ± 0.04D	0.20 ± 0.04D
191	0.61 ± 0.06B	0.82 ± 0.03A	0.60 ± 0.06B	0.12 ± 0.03D	0.06 ± 0.01E	0.11 ± 0.03D

Note: Different letters represent significant differences at the $p < 0.05$ probability level.

the family, it has been hypothesized that apomixis might be controlled by the same set of genes wherever it occurs. However, ASGR showed no relevant large-scale collinearity and/or synteny with reference genomes (rice, *Setaria*, and *Sorghum*) (Galla et al., 2019).

To the present, the genetic basis and origins of apomixis are still unclear (Terzaroli et al., 2023). Even more, these authors highlighted the importance of the epigenetic phenomenon, which is the link between the environment, the genes, and their asynchronous expression, and considered that epigenomics and transcriptome investigation could represent a new holistic approach to analyze apomixis mechanisms and its origin further. Particularly in buffel grass, Rathore et al. (2020) found that hypomethylation of retro-element Gypsy163 might be responsible for the initiation of apomictic seed development, while Ke et al. (2021) identified different transcriptional profiles between parthenogenetic and sexual eggs and suggested functional roles for a few transcription factors in promoting natural parthenogenesis.

As can be noticed, and as postulated by Kumar (2017), the epigenetic control of apomixis is an emerging theory, with growing evidence (Karunarathne et al., 2020; Mateo de Arias, 2015; Podio et al., 2014; Rios et al., 2013; Soliman et al., 2021) in several species. Epigenetic changes and alterations in gene expression, particularly through DNA methylation, provide a means to achieve desirable phenotypic plasticity and adaptive advantages (Kumar et al., 2019). Thus, the maintenance of a residual sexual pathway provides an opportunity for genetic diversification and evolution via sexual recombination (Chapman et al., 2003; Hörandl & Hojsgaard, 2012).

Our results confirm the facultative nature of buffel grass (Negawo et al., 2020; Quiroga et al., 2013; Sharma et al., 2014), although the proportions of the sexual reproductive mode in the assessed genotypes were lower than 10%, as reported by Hussey et al. (1991) and Hignight et al. (1991). These low proportions, however, represent the ability of facultative apomictic buffel grass genotypes to generate low levels of genetic variation through rare events of sexuality, as reported by Hignight et al. (1991) for buffel grass and by Reutemann et al. (2022) for *Paspalum* sp. Furthermore, differences among flowering periods, although non-statistically

significant, were noticed, and a decreasing trend from BF toward EF was observed. These results agree with those reported by Hussey et al. (1991) for buffel grass and with those reported by Rebozzio et al. (2011) and Rios et al. (2013) for *Paspalum* sp. The authors noticed a higher apospory expression when tillers developed during the hottest period of the year (i.e., PF) and higher sexual expression in the spring and fall (i.e., BF and EF, respectively). Since we did not find a significant influence of bioclimatic variables on the reproductive mode and since the influence of changing environmental conditions on phenotypic expression of apospory/sexuality has been scarcely explored in buffel grass (Gounaris et al., 1991; Hussey et al., 1991) under field conditions, further studies are necessary to more accurately understand the behavior of this trait.

A greater variation in PSE was observed when the analysis considered periods of flowering rather than genotypes (Table 3). In facultative apomictic bahiagrass, the potential for sexuality may also be exploited to increase genetic diversity, especially at the beginning or at the end of the flowering season, when facultative apomicts exhibit the greatest potential for sexual reproduction (Rebozzio et al., 2011; Reutemann et al., 2022; Rios et al., 2013). According to the latter and in agreement with our results of the reproductive mode, the greatest PSE was observed at BF, being statistically different from the other two flowering periods assessed and with significant influence of all bioclimatic variables considered, except for cumulative precipitation. At BF (Table 1), thermal time showed the lowest value (444.4°C day^{-1}), whereas cumulative radiation (323.07 W (m^2)$^{-1}$) and average photoperiod length (14.54 h day^{-1}) showed their highest values.

Furthermore, the results of the PLS regression analysis (Figure 2) confirmed a significant and positive incidence of average photoperiod length on PSE, which showed its highest value at BF (Table 3), coincidentally with the sexual pathway behavior. As reported by Klatt et al. (2016), this result would be due to the extended photoperiod, a stressor that triggers megasporogenesis and the development of MES. By contrast, Hussey et al. (1991) reported that the highest MES frequency occurred in photoperiods of less than 13.2 h day^{-1} during spring; however, those authors found no significant inci-

dence of photoperiod and suggested that other environmental factors could influence the expression of sexuality. These differences in the results may be due to the different statistical analyses performed and/or differences among experimental conditions (growth chambers vs. field conditions).

In a study involving *Paspalum malacophyllum*, Hojsgaard et al. (2013) reported the existence of competition between sexual and apomictic pathways, from megasporogenesis to offspring establishment. In such competition, the apomictic pathway remained functional and gave rise to clonal progeny. Recently obtained progenies will be molecularly assessed to determine their F1 hybrid condition, since it is not possible to perform flow cytometry screen of seeds in buffel grass (Kharrat-Souissi et al., 2013; Matzk et al., 2000) to determine the still unclear real efficiency of the sexual reproductive mode and PSE in this species.

Self-incompatibility is a genetic mechanism of hermaphroditic plants to prevent inbreeding after self-pollination (Rohner et al., 2023) and has significant effects on population structure, potential diversification, and evolutionary consequences (Yang et al., 2008). This trait is frequently estimated as the seed set of randomly selected OP and SP panicles (Acuña et al., 2007, 2009; Conde-Lozano et al., 2011; Hignight et al., 1991; Shafer et al., 2000). The evaluated genotypes showed higher seed fertility in OP than SP (Table 3), which is consistent with the outcrossing and pseudogamous nature of buffel grass (Hanselka et al., 2004; Shafer et al., 2000). In addition, there was a considerable variation in seed set among the OP genotypes, as reported by Hignight et al. (1991), Shafer et al. (2000), and Conde-Lozano et al. (2011), although those authors did not consider the environmental influence on this trait.

It is known that environmental conditions significantly impact seed set in both pollination methods (Gutterman, 2000; Kissling et al., 2006; Krishnan & Rao, 2005; Zilli et al., 2018). However, there are no previous reports regarding the seasonal variation of seed set in buffel grass. Our results showed that seed set depended mainly on environmental conditions rather than on genotypic effects, as reported by Krishnan and Rao (2005). Furthermore, the significant influence of bioclimatic variables was confirmed, and the PLS analysis evidenced that photoperiod, thermal time, and cumulative precipitations, which showed their greatest values at BF and PF, favored OP seed-set, as reported by Hanselka et al. (2004), regardless of the year of evaluation, unlike results reported by Zilli et al. (2018) in *Paspalum notatum*. This result could be due to the lack of conditions restricting crop growth and development, and the decrease of cumulative precipitation during the reproductive stage, which provided dry conditions, favoring seed maturity.

As reported by Klatt et al. (2016) for *Ranunculus auricomus*, we found that photoperiod, which was the longest at BF, acted as an environmental stressor that promoted the potential expression of sexual reproduction in facultative apomictic buffel grass genotypes at the beginning of the flowering season. In addition, the OP seed set showed greater values at BF and PF than at EF, which enhances the probability of this species to propagate through recombinant individuals.

5 | CONCLUSIONS

In the current climate change context, understanding the genetic basis and identifying the environmental influence on the reproductive behavior of a species is fundamental for diversity conservation and system sustainability. In this study, we confirmed that the reproductive performance of the assessed buffel grass genotypes was influenced by several bioclimatic variables. These genotypes behave mainly as outcrossers, and their seed fertility under open pollination was explained by the prevailing cumulative precipitations, average photoperiod length, and thermal time during the flowering season. They also showed the highest potential for sexual reproduction at the beginning of the flowering season, which was promoted by a prolonged average photoperiod. Therefore, we conclude that at the beginning of flowering, the studied genotypes have a remarkable potential as female parent for hybridizations programs in *C. ciliaris*. To our knowledge, this is the first study to explore the influence of environmental conditions on the reproductive mode, potential sexual expressivity, and seed fertility throughout the flowering season for *C. ciliaris* genotypes under field conditions.

AUTHOR CONTRIBUTIONS
Miguel A. Sánchez: Data curation; formal analysis; investigation; methodology; software; writing—original draft; writing—review and editing. **Cecilia I. Bruno**: Data curation; formal analysis; methodology; software; supervision; writing—original draft; writing—review and editing. **Mariana P. Quiroga**: Conceptualization; investigation; methodology; supervision; writing—original draft; writing—review and editing. **Karina A. Grunberg**: Conceptualization; funding acquisition; investigation; methodology; project administration; supervision; writing—original draft; writing—review and editing. **Sabrina M. Griffa**: Conceptualization; funding acquisition; investigation; methodology; project administration; supervision; writing—original draft; writing—review and editing.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

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