

Seasonal expression of apospory in bahiagrass

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Introduction

Flowering plants can reproduce sexually (outcrossing and/or selfing) and/or asexually. Sexual reproduction implies the successful completion of meiosis and double fertilization for the formation of both the embryo and the endosperm. In contrast, gametophytic apomixis is an asexual mode of reproduction through seeds, that involves parthenogenetic embryo development from a cytologically unreduced egg cell (2n). Apospory is the process by which unreduced gametophytes are formed after a series of mitotic divisions of somatic cells (2n) in the ovary. This occurs independently from the sexual meiotic process; and therefore, both sexual and apomictic pathways may coexist simultaneously.

Apospory is inherited in bahiagrass (*Paspalum notatum* Flügge) as a single dominant Mendelian factor with distorted segregation (Martínez et al. 2001), and its degree of expression was reported to vary throughout the flowering season in *P. cromyorrhizon*, a close relative of bahiagrass (Quarin 1986). Bahiagrass is a perennial warm-season grass widely used for forage and utility turf in the south-eastern US due to its persistence in sandy, infertile soils. Diploid races reproduce sexually and are highly self-incompatible (Acuña et al. 2007), while polyploids are classified as pseudogamous apomicts (pollination is required) (Quarin 1999). Sexual tetraploid genotypes have been experimentally created (Quesenberry and Smith 2003; Quesenberry et al. 2010) and successfully used in crosses (Acuña et al. 2009). Cytogenetic analysis has been used to determine the mode of reproduction in bahiagrass (Martínez et al. 2001; Acuña et al. 2007). At anthesis, sexual plants produce spikelets having only a single *Polygonum* type

meiotic embryo sac (SES), characterized by bearing the egg apparatus close to the micropyla, a large binucleated central cell and a group of antipodal cells at the chalazal end (Figure 1a). Highly apomictic plants produce ovules having single or multiple aposporous embryo sacs (AES), which present the egg apparatus and a central cell with 2 polar nuclei, and no antipodal cells (Figure 1b). Some tetraploid bahiagrass races are also able to produce ovules that have the sexual meiotic megasporocyte together with one or more aposporous sacs (AES+SES), and these plants are classified as facultative apomictic. The objective of this study was to characterize the reproductive mode of 5 wild dwarf bahiagrasses, a highly apomictic hybrid (Acuña et al. 2009) and cv. Argentine at different times during the flowering season and under different nitrogen (N) fertilizer rates.

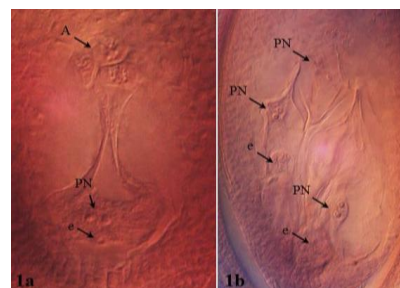


Figure 1. Types of embryo sacs: (a) ovule bearing an SES; (b) ovule having at least 3 AES. A = antipodals, e = egg cell, and PN = polar nuclei. Magnification: x 312.5.

Methods

The study was conducted on a bahiagrass sward planted in the fall of 2011 at the Agronomy Forage Research Unit, near Gainesville, FL, USA. The experimental design was a randomized complete block design in a strip split-split-plot arrangement with 3 replications. Main

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plots consisted of 8 bahiagrass genotypes: 5 wild dwarf bahiagrasses (3 Fpen 7; 3 Fpen 9; Fldw 5-1; Fldw 6-4; and Fldw 6-5), 2 highly apomictic hybrids (Hyb 3; and Hyb 14), and cv. Argentine. Subplots were the dates of plant defoliation at 10 cm (May and June) and sub-subplots were different N fertilizer rates (0, 60 and 120 kg N/ha) applied randomly using ammonium nitrate immediately after mowing the plots. Inflorescences were collected at anthesis at 3 different times during the flowering season: (a) spring: before mowing the plots (only the facultative apomicts were flowering); (b) summer; and (c) fall. In the summer and fall inflorescences were collected only from plots mowed in May. Additionally, only the genotypes 3 Fpen 9, Fldw 6-5, Hyb 3 and Argentine were analyzed in the summer and fall. For each embryo sac observation, 2 different inflorescences at anthesis were fixed in FAA (18 Ethanol 70%:1 Formaldehyde 37%:1 glacial acetic acid). Pistils were dissected out of the spikelets and cleared following the methodology described by Young et al. (1979). At least 20 ovules per sample were observed using a differential interference contrast microscope.

Results and Discussion

The 5 dwarf genotypes were classified as facultative apomicts, since all of them produced florets having AES, SES or both AES+SES in the same ovule in the inflorescences collected in the spring. The proportion of AES varied (29–55%) among the dwarf plants. All genotypes showed a high number of ovules bearing AES+SES. Ovules bearing SES were produced with a lower frequency, ranging from 12 to 20%. Overall, the production of aborted or immature embryo sacs (AbES) was low, except for the genotype Fldw 6-5 (20%). Nitrogen fertilizer did not affect apospory in any of the genotypes; therefore results from the 3 N rates were averaged within season (summer and fall) for each genotype. Genotypes Hyb 3 and Argentine bahiagrass reproduced by apomixis as reported by Acuña et al. 2009. These genotypes were classified as highly apomictic because more than 90% of the embryo sacs produced in either the summer or fall were AES, while the remainder were AbES (Figure 2).

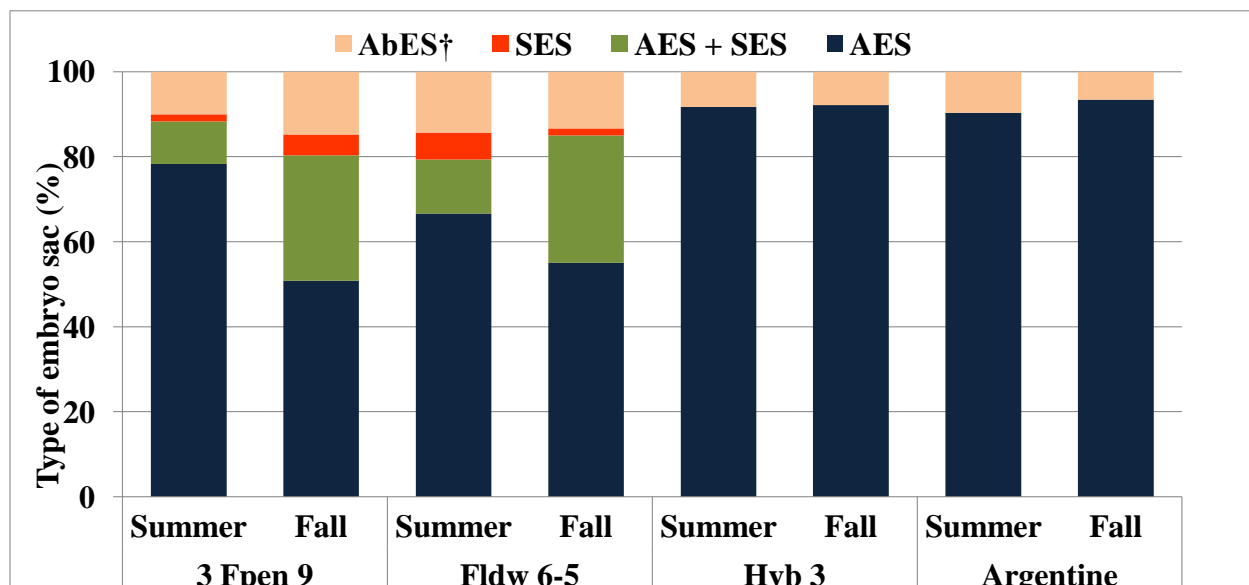


Figure 2. Types of embryo sac observed in 2 facultative apomictic (3Fpen9 and Fldw6-5) and two obligate apomictic (Hybrid 3 and Argentine) bahiagrass genotypes at two different stages of the flowering season.

†AbES: aborted or immature embryo sacs; SES: meiotic embryo sacs; AES: aposporic embryo sacs; AES+SES: AES and SES embryo sacs in the same ovule.

The two facultative apomictic dwarf plants performed similarly in their modes of reproduction in the summer and fall (Figure 2). Apospory was expressed in greater proportion in the summer (60–80% of the embryo sacs were AES); however, in the fall the production of AES+SES increased, reaching similar values as in the

spring. Therefore, the potential for apomictic reproduction increases as the plants reach peak flowering (summer). Hence, the environmental conditions that affect flowering may also be involved with the expression of apospory, as Quarin (1986) proposed for *P. cromyorrhizon*.

Conclusion

The 5 wild dwarf genotypes behaved as facultative apomictic and the expression of apospory varied throughout the flowering season. The potential for apomixis (AES) increased as the plants reached their peak flowering (summer); however, the potential for sexual reproduction (AES+SES and SES) increased in the spring and fall. Apospory was consistently expressed throughout the flowering season in the highly apomictic genotypes Hyb 3 and Argentine. These results have implications for seed harvesting.

In general, the dwarf genotypes produced more AbES than the highly apomictic plants, so a lower seed set may be expected from them. Considering that each embryo sac had the same opportunity to develop a viable seed, time of seed harvest can be used to manipulate objectives in a breeding program. When new genetic variability is desired, seed should be harvested in the spring or fall. In contrast, when harvest occurs during peak flowering, progeny will be highly uniform. As N fertilizer had no impact on the expression of apospory in these genotypes, application of fertilizer to improve seed production can proceed without concern about likely impact on reproductive pathway.

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