

Tetraploid bahiagrass hybrids: breeding technique, genetic variability and proportion of heterotic hybrids

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Abstract Manipulating apomixis for fixing tetraploid bahiagrass (*Paspalum notatum* Flüggé) hybrids exhibiting superior agronomic characteristics would be a valuable tool for the genetic improvement of this species. The objectives were to create a second generation of hybrids by crossing sexual first-generation tetraploid hybrids (FGTH) and unrelated apomictic FGTH or ecotypes, determine the segregation for mode of reproduction, and estimate the resulting genetic variation and heterosis for several agronomic characteristics. The segregation for mode of reproduction was analyzed using mature embryo sac observations. Field measurements and visual ratings were used to evaluate the growth habit, production of inflorescences, cool-season growth and freeze resistance of hybrids. A ratio of 4.6:1 between sexual and apomictic hybrids was observed. Only 3% of the progeny was classified as highly apomictic. The low

proportion of highly apomictic progeny restricts the feasibility of this breeding approach. Large genetic variation was observed among hybrids for growth habit, cool-season growth and freeze resistance. The proportion of hybrids exhibiting heterosis was high for plant height, cool-season growth, and freeze resistance. The tetraploid bahiagrass germplasm contains a large diversity for traits of agronomic importance that can be exploited for the genetic improvement of this species.

Keywords Apomixis · Heterosis · *Paspalum* · Polyploidy

Introduction

Approximately 125 grass species form their seeds by an asexual process called apomixis (Bashaw and Hanna 1990). This characteristic offers a unique opportunity for developing and using superior genotypes. Seed of any superior obligate apomict could be increased through open-pollination for an unlimited number of generations without loss of vigor or change in genotype (Hanna and Bashaw 1987).

Apomixis is the predominant mode of reproduction among the polyploid germplasm of *Paspalum* (Quarin 1992). Bahiagrass, *Paspalum notatum* Flüggé, has become one of the most economically important species of this genus mainly because of its use as forage and utility turf (Blount and Acuña 2009). This

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species includes polyploids that reproduce by apomixis and diploids that reproduce sexually (Burton 1948; Burton 1955). Apomixis in bahiagrass includes the formation of unreduced embryo sacs from nucellar cells (apospory), the parthenogenetic development of embryos, and the development of the endosperm following fertilization of the polar nuclei (pseudogamy). Apospory in this species is inherited as a single dominant Mendelian factor with distorted segregation (Martínez et al. 2001). This factor is located in a genomic region characterized by suppression of recombination and preferential chromosome pairing (Martínez et al. 2003; Stein et al. 2004; Stein et al. 2007).

In nature, the tetraploid ($2n = 4x = 40$) cytotypes of bahiagrass are predominately facultative or obligate apomictic, and produce normally reduced pollen ($n = 2x = 20$) (Gates et al. 2004). All tetraploid cultivars released in the USA, Japan and Australia, were considered to be superior apomictic ecotypes originally collected as plant introductions from Brazil, Argentina, Paraguay and Uruguay (Blount and Acuña 2009). Sexual tetraploids have been generated by treating both diploid ($2n = 2x = 20$) seed and tissue cultured calluses with chromosome duplication treatments (Forbes and Burton 1961; Quarín et al. 2001; Quesenberry et al. 2010). Crosses between induced sexual tetraploid clones, used as female parents, and apomictic tetraploid clones, used as pollen donors, resulted in the release of extensive genetic variability for traits of agronomic importance among the resulting hybrids (Acuña et al. 2009). When the progeny were classified by mode of reproduction, 80% of the hybrids were sexual, 11% were highly apomictic, and 9% were facultative apomictic. Continued improvement should theoretically be possible by repeated crossing of superior sexual plants with superior apomictic male pollinators. Although the “apospory locus” has a pleiotropic lethal effect with incomplete penetrance (Martínez et al. 2001), the proportion of apomictic hybrids is expected to remain within a range that makes this breeding approach feasible across cycles of hybridization.

High genetic variability for multiple traits including growth habit, photoperiod sensitivity, and freeze resistance has been observed in both the diploid and tetraploid bahiagrass germplasm (Blount and Acuña 2009). This natural variability can be exploited

through appropriate breeding techniques to generate improved hybrids and populations. For example, a diploid population with superior cool-season growth and cold resistance was generated using four cycles of restricted recurrent phenotypic mass selection, and released as “UF-Riata” in 2007 (Blount and Acuña 2009). The tetraploid germplasm offers the possibility of manipulating apomixis for fixing hybrids with superior agronomic characteristics. Although continuous breeding may be possible in this group, the genetic variability for agronomic traits with quantitative inheritance is expected to be reduced by each cycle of hybridization and selection.

The objectives of this research were (1) to create a second generation of tetraploid hybrids (SGTH) by crossing sexual first generation tetraploid hybrids (FGTH) and unrelated apomictic FGTH or natural hybrids, (2) determine the segregation for mode of reproduction, (3) estimate the resulting genetic variation, and (4) determine the proportion of hybrids exhibiting mid-parent heterosis for growth habit, cool-season regrowth, production of inflorescences and freeze resistance.

Materials and methods

Plant material and crosses

Crosses were made between sexual and apomictic tetraploid clones during the summer 2006 to generate a segregating bahiagrass population named second generation of tetraploid hybrids (SGTH). Eleven sexual tetraploid clones were used as female parents (Table 1). These sexual parents, named first generation of tetraploid hybrids (FGTH), were hybrids generated at the University of Florida by crossing induced tetraploids (derived from seeds of the diploid cultivar Tifton 9) and the apomictic tetraploid clones, Argentine and Tifton 7 (Acuña et al. 2009). These 11 FGTH were selected for this study because they were identified as sexual and cross-pollinated, based on 2 years of observations, and were selected out of several hundred clones based on growth habit, cool-season regrowth and freeze resistance (Acuña et al. 2009). Six highly apomictic clones were used as pollen donors (Table 1). Four of them were also FGTH generated by crossing induced sexual and apomictic tetraploids, identified as highly apomictic

Table 1 Seed set, germination, reproductive efficiency, and number of hybrids resulting from crosses between sexual and apomictic tetraploid clones of bahiagrass

Table 1 Seed set, germination, reproductive efficiency, and number of hybrids resulting from crosses between sexual and apomictic tetraploid clones of bahiagrass	Cross			Seed set ^a (%)	Germination (%)	Reproductive efficiency ^b (%)	Number of hybrids
	Sexual female	Apomictic male	Number				
	FL-83	FL-13	1	32	98	32	424
	FL-137	FL-13	2	51	89	46	512
	FL-99	FL-3B	3	35	99	35	256
	FL-14	FL-93	4	44	80	35	374
	FL-16	FL-93	5	26	70	18	219
	FL-41	FL-25	6	23	83	19	154
	FL-3C	Argentine	7	39	97	38	165
	FL-7	Tifton 7	8	24	92	22	192
FL-62	Tifton 7	9	26	72	19	169	
FL-47	Tifton 7	10	23	84	20	87	
FL-16	Argentine	11	13	66	9	92	
FL-20	Argentine	12	20	59	12	56	
Average			30	82	25		

^a Seed set: percentage of obtained seed from the total number of pollinated florets

^b Reproductive efficiency: percentage of obtained plants from the total number of pollinated florets

(Acuña et al. 2009), and selected based on superior spreading, cool-season regrowth and freeze resistance. The other two apomictic parents were the cultivar Argentine, which is the best adapted tetraploid ecotype to southeastern USA, and the experimental hybrid Tifton 7. Argentine and Tifton 7 were also classified as highly apomictic by cytological analysis and field progeny tests (Acuña et al. 2007). Cross combinations were chosen to include maximum diversity among the parents. This was accomplished by crossing unrelated parents.

Crosses were made by enclosing one inflorescence from the sexual female and one or two inflorescences from the apomictic male in a glassine bag prior to anthesis. Care was taken to select inflorescences at the same stage of maturity. All bags were shaken each day during anthesis. Five days after anthesis the inflorescences from the apomictic parent were removed from the bags, leaving only the inflorescences from the sexual parent until the seed mature. At maturity, each head from the sexual parents was threshed separately, the number of florets was counted, empty florets were removed, and the number of florets containing caryopses was determined.

Seeds were scarified using concentrated sulfuric acid for 10 min, and were sown in flats containing sterile germination medium in February 2007. Individual seedlings were later transplanted to seedling flats in a greenhouse. Plants were transplanted into a field located at the Agronomy Forage Research Unit

near Hague, Florida on 11 May 2007. The soil classification at this location was a loamy, siliceous, subactive, thermic, Arenic Endoaquult. Parents were asexually propagated in the greenhouse and transplanted into the field with the progeny. While the apomictic parents were propagated by seeds, sexual parents were vegetatively propagated using short pieces of rhizomes containing the apical meristems. Progeny from each cross were planted in 55-plant rows where individual plants were spaced 1 × 1 m. Ten replications of each of the two parents involved in the specific cross were planted in the row next to the progeny. The field was fertilized with 290 kg ha⁻¹ of 21–3.1–11.6 (N–P–K) in June 2007.

Progeny evaluation

Mode of reproduction

Two hundred and eleven plants were selected from 2,700 total generated plants. Selection was based on good initial vigor, i.e., growth exhibited during the vegetative stage in 2007. This subsample was used to estimate the proportion of apomictic and sexual progeny. This selected subsample was used for the analysis of mode of reproduction to reduce the probability of including progeny resulting from accidental self pollination among the evaluated plants.

Inflorescences from this group of selected plants were fixed at anthesis (when the embryo sacs are

usually fully developed) in FAA (18 Ethanol 70%:1 Formaldehyde 37%:1 glacial acetic acid). Pistils were dissected out of the florets and cleared using the method of Young et al. (1979). Ovules were observed using a differential interference contrast microscope. Inflorescences were fixed at peak flowering, between 10 July and 10 August 2009, because previous research indicated that the maximum expression of apomixis occurred at this stage of development (Quarin 1986).

A minimum of 20 ovules were observed from at least two different inflorescences. Plants bearing ovules with single embryo sacs containing the egg apparatus, the bi-nucleated central cell, and a mass of antipodals at the chalazal end were classified as sexual. In contrast, plants bearing ovules with multiple or single embryo sacs with the egg apparatus, the central cell, no antipodals, and variable size and position, were classified as apomictic. Plants producing ovules with either reduced or unreduced embryo sacs were classified as facultative apomictic.

Field observations

Plant diameter was estimated using the average between the longest and the shortest diameter of a given plant on 2 October 2007, and 26 September 2008. Plant height was measured from the base of the plant to the top of the canopy on 21 September 2007 and 12 September 2008. The number of inflorescences per plant was counted on 22 September 2007.

On 23 September 2007 all plants were clipped to approximately 6-cm above the soil level and regrowth was visually estimated on 28 October 2007, and 6 May 2008 using a 1–5 scale, where 1 = plants showing the lowest amount of herbage and 5 = plants showing the highest amount of herbage. Plants were clipped on 19 September 2008 and regrowth was estimated on 7 November. Also, freeze resistance was visually estimated on 21 November 2008 after one freeze event on 19 November, with temperature of -6°C , using a 1–5 scale, where 1 = the least freeze resistant, and 5 = the most freeze resistant plant.

Statistical analysis

Chi-square tests were used to compare the observed apomixis/sexuality segregation ratio with expected

ratios and with previously reported ratios. Field observations were analyzed using PROC GLM of PC SAS (SAS Institute 2004) as a completely randomized design. Hybrids were considered the experimental units for comparisons among families. When significant differences among families were detected for one variable, the Waller–Duncan Test was used for mean separations. Unless otherwise stated in the text, all differences refer to significance at $P < 0.05$.

Broad sense heritability (H^2) estimates were calculated using the following formula:

$$H^2 = \frac{\sigma_p^2 - \sqrt{(\sigma_{sp}^2 * \sigma_{ap}^2)}}{\sigma_p^2}$$

where σ_p^2 equals the phenotypic variance among the progeny, σ_{sp}^2 equals the phenotypic variance among clonal replications of the sexual parent, and σ_{ap}^2 equals the phenotypic variance among replications of the apomictic parent. σ_p^2 includes the additive, dominance, and epistatic genetic variance, variation due to interactions between genotypes and environment, and variation due to environmental effects. The environmental variation was estimated based on the variation among clonally propagated parents. Variances and means were obtained using Proc Univariate of SAS.

The proportion of hybrids exhibiting positive mid-parent heterosis was calculated for plant diameter, plant height, number of inflorescences, spring and fall regrowth, and freeze resistance. Mid-parent heterosis (MPH) was calculated for each hybrid using the following formula:

$$\text{MPH} (\%) = \frac{(F_1 - \text{MP})}{\text{MP}} \times 100$$

where F_1 equals the performance of hybrid, and MP equals the average performance of parents per se (parent 1 + parent 2)/2.

Results

Hybridization efficiency

In 2006, 2,700 progeny were generated using 12 combinations of selected sexual and apomictic

bahiagrass clones. Parental clones were selected based on their mode of reproduction (highly sexual or highly apomictic) and superior agronomic characteristics including cool-season regrowth, spreading and leaf tissue freeze resistance (Acuña et al. 2009). Sexual clones were used as female parents and highly apomictic clones as pollen donors. The average seed set was 30% varying from 13 to 51% (Table 1). Great variation was observed among crosses for germination. The average germination was 82% varying from 59 to 99%. The average reproductive efficiency resulting from these crosses was 25% varying from 9 to 46% indicating that the outcome from this type of hybridization is highly dependent on the genotypes selected as parents.

Segregation for mode of reproduction

Two hundred and eleven progeny were selected based on their superior vigor to study the segregation for mode of reproduction. The use of this approach was expected to reduce the probability of including progeny resulting from self-pollination of sexual parents in the study. Progeny from self-pollination that could be present among hybrids were expected to have less vigor. Although different numbers of progeny were selected from each family, all families were represented in this group (Table 2). One hundred and seventy-three plants were classified as

Table 2 Bahiagrass tetraploid progeny classification for method of reproduction based on embryo sacs observations

Cross ^a	Analyzed progeny	Apomictic	Facultative	Sexual
1	22	0	3	19
2	33	0	4	29
3	22	0	3	19
4	43	2	12	29
5	23	3	3	17
6	12	1	0	11
7	4	0	2	2
8	19	1	1	17
9	16	0	0	16
10	3	0	0	3
11	2	0	0	2
12	12	0	3	9
Total	211	7	31	173

^a See Table 1 for cross identification

sexual because only single reduced embryo sacs were observed in their ovules. Seven were classified as highly apomictic because aposporous embryo sacs were observed in no less than 65% of their ovules. The remaining 31 plants were classified as facultative apomictic because aposporous embryo sacs were observed in no more than 30% of their ovules.

Genetic variability and proportion of heterotic hybrids

Growth habit and production of inflorescences

Plant diameter and plant height were measured in 2007 and 2008 to characterize the growth habit of the progeny. Significant differences were observed among families for plant diameter in both years. A range of family means from 28 to 42 cm in diameter was observed in 2007 and from 35 to 60 cm in 2008 (Table 3). The highest variability resulted from cross 5 in 2007 and cross 6 in 2008 (Table 3). The average broad sense heritability for plant diameter was 0.8 varying from 0.47 to 0.97 (Table 4). Positive mid-parent heterosis for plant diameter was exhibited by 15% of the hybrids in 2007 and 13% in 2008

Table 3 Plant diameter and plant height for progeny of 12 combinations of sexual and apomictic clones

Cross ^a	Plant diameter				Plant height			
	2007		2008		2007		2008	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	33	8.9	50	13.9	40	8.6	51	8.6
2	42	11.0	59	22.6	34	7.7	48	8.9
3	35	8.0	47	11.2	43	7.9	53	31.6
4	34	8.4	50	12.4	47	8.0	63	9.0
5	40	14.4	60	15.7	40	9.4	52	21.2
6	40	7.7	60	39.2	40	8.2	48	11.1
7	28	8.3	35	15.6	40	7.9	51	15.4
8	40	8.7	59	28.4	36	7.7	48	10.7
9	37	9.4	49	14.6	36	8.4	47	10.2
10	37	10.0	54	20.3	32	7.6	42	11.8
11	38	7.8	59	33.0	36	7.5	40	11.5
12	37	6.4	59	10.6	43	8.1	57	8.8
MSD ^b	2.0		4.2		1.7		3.0	

^a See Table 1 for cross identification

^b Minimum significant difference, Waller–Duncan means separation procedure

Table 4 Broad-sense heritability estimates for several characteristics measured for 12 combinations of sexual and apomictic bahiagrass tetraploid clones

Cross ^a	Number of inflorescences	Height	Diameter	Spring regrowth	Fall regrowth	Freeze resistance
1	0.40	0.78	0.82	0.46	0.49	0.66
2	0.66	0.61	0.85	0.71	0.49	0.68
3	0.22	0.95	0.59	0.86	0.78	0.52
4	0.65	0.90	0.74	0.63	0.66	0.85
5	0.82	0.93	0.86	0.41	0.65	1.00
6	0.40	0.78	0.97	0.72	0.47	0.68
7	0.50	0.90	0.80	0.75	0.86	0.74
8	0.19	0.71	0.93	1.00	0.79	1.00
9	0.27	0.65	0.80	0.63	0.56	0.25
10	0.36	0.80	0.83	0.56	0.52	1.00
11	0.55	0.84	0.94	0.31	0.49	1.00
12	0.68	0.70	0.47	0.65	0.77	0.81
Mean	0.48	0.79	0.80	0.64	0.63	0.77

^a See Table 1 for cross identification

Table 5 Number of inflorescences, spring and fall regrowth, and freeze resistance of progeny resulting from 12 combinations of sexual and apomictic bahiagrass tetraploid clones

Cross ^a	Number of inflorescences		Spring regrowth		Fall regrowth		Freeze resistance	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	13	12.4	2.0	0.9	2.6	1.0	1.8	0.8
2	21	15.4	2.8	1.0	2.9	0.9	2.0	0.8
3	26	15.7	2.7	1.1	3.1	1.0	2.0	0.8
4	28	17.3	3.5	1.2	3.3	1.0	2.6	1.0
5	31	17.2	2.2	0.9	2.9	0.9	1.8	0.7
6	22	14.6	2.3	1.0	2.8	0.8	1.7	0.7
7	11	11.6	2.0	0.9	2.6	0.9	2.0	1.0
8	16	18.1	2.7	0.9	3.0	0.7	2.0	0.8
9	18	18.5	2.3	0.9	2.8	0.8	1.7	0.6
10	25	17.7	2.0	0.9	2.2	0.8	1.7	0.6
11	39	14.0	1.7	0.6	2.1	0.6	1.6	0.5
12	36	13.3	3.4	0.9	3.0	0.7	2.8	1.1
MSD ^b	3.2		0.2		0.1		0.2	

^a See Table 1 for cross identification

^b Minimum significant difference, Waller–Duncan means separation procedure

(Table 6). The proportion of heterotic hybrids for this trait varied among families from 3 to 30% in 2007 and from 4 to 30% in 2008. Significant variation was also found among families for plant height in 2007 and 2008. Family means for height varied from 32 to 47 cm in 2007 and from 40 to 63 cm in 2008 (Table 3). The greatest amount of variability was observed for cross 5 in 2007 and for cross 3 in 2008 (Table 3). The average broad sense heritability for plant height was 0.79 varying among families from 0.61 to 0.95. In addition, positive mid-parent heterosis for plant height occurred for 48% of hybrids in 2007 and 34% in 2008 (Table 6). The proportion of

heterotic hybrids for this trait varied among families from 17 to 96% in 2007 and from 9 to 81% in 2008.

Since seed production is considered an important agronomic characteristic of bahiagrass, the total number of inflorescences per plant was counted at the end of the 2007 growing season. The mean number of inflorescences produced varied from 13 to 39 among families (Table 5). The highest variability was obtained from cross 9. Broad sense heritability varied from 0.19 to 0.82 among families, while the average was 0.48 (Table 4). Only 8% of hybrids exhibited mid-parent heterosis varying from 2 to 27% among families (Table 6).

Table 6 Proportion of bahiagrass hybrids exhibiting positive mid-parent heterosis for several phenotypic characteristics, resulting from 12 combinations of sexual and apomictic tetraploid clones

Cross ^a	Plant diameter (%)		Plant height (%)		Number of inflorescences (%)	Spring regrowth (%)	Fall regrowth (%)		Freeze resistance (%)
	2007	2008	2007	2008			2007	2008	
1	7	8	31	19	2	3	13	0	17
2	15	17	17	9	9	23	19	24	18
3	8	5	29	26	2	51	38	27	71
4	8	4	81	79	14	54	50	24	50
5	30	16	56	40	17	7	69	21	71
6	27	17	79	24	7	40	70	16	8
7	3	6	88	61	3	24	55	18	26
8	29	23	51	32	9	17	17	72	15
9	24	20	42	20	5	38	61	26	7
10	22	30	29	30	6	30	21	9	6
11	16	16	68	36	27	5	14	4	53
12	4	26	96	81	22	85	81	28	52
Overall	15	13	48	34	8	29	38	23	32

^a See Table 1 for cross identification

Cool-season growth and freeze resistance

The 12 families included in this study were determined to be significantly different in terms of plant regrowth during fall 2007, spring 2007 and fall 2008. The largest variability for spring regrowth was contained in family 4, while the lowest was present in family 11 (Table 5). Broad sense heritability estimates varied greatly among families having an average of 0.64 (Table 4). In addition, 29% of the hybrids exhibited heterosis for this trait varying from 3 to 85% among families (Table 6). Progeny within family 1, 3 and 4 exhibited the greatest amount of variability for fall regrowth considering 2007 and 2008 data (Table 5). The average broad sense heritability for fall regrowth was 0.63 varying from 0.47 to 0.86 (Table 4). Positive heterosis was observed for 38% of the hybrids in 2007 and 23% in 2008. The proportion of heterotic hybrids varied among families from 13 to 81% in 2007 and from 0 to 72% in 2008.

Significant differences were also observed among these 12 families for freeze resistance in 2008. The greatest amount of variability for this characteristic resulted from cross 12, while the lowest variation resulted from cross 11 (Table 5). The average broad sense heritability was 0.77, varying from 0.25 to 1.0 (Table 4). In addition, heterosis was observed for 32% of the hybrids varying from 6 to 71% among the 12 families.

Discussion

The opportunity of fixing superior seed-propagated genotypes via apomixis and the information generated in the last decade concerning the genetic control of apomixis have encouraged new attempts for the genetic improvement of apomictic bahiagrass as forage and turf. This research shows that it is feasible to generate a segregating population by crossing selected sexual FGTH and apomictic bahiagrass clones. In fact, SGTH can be created more efficiently than FGTH mainly because of the differences in fertility and vigor of the involved female parents. While sexual FGTH are superior genotypes selected from an original segregating population and have normal fertility, induced sexual tetraploid clones usually have reduced fertility and low vigor.

The ratio between sexual and apomictic SGTH was of 4.6:1, which was different from the 1:1 segregation ratio [$\chi^2 = 86.4$, $P(1 \text{ df}) < 0.001$] expected for a monogenic tetrasomic inheritance with apospory as a dominant trait. However, the observed segregation pattern was not different from the 4.3:1 ratio [$\chi^2 = 0.5$, $P(1 \text{ df}) = 0.48$] observed for the FGTH. These results would indicate that although there is a strong distortion with an excess of sexual progeny, the segregation patterns for apomixis remain constant through hybridization cycles. However, the proportion of highly apomictic progeny decreased from 11%

in the FGTH to only 3% in the SGTH, and the proportion of facultative apomictic increased from 9% in the FGTH to 15% in the SGTH. These findings would indicate that the probability of finding highly apomictic progeny decreases through hybridization cycles. This variable expressivity between generations could be related with other genes playing a minor role in the expression of aposporous apomixis. Environmental differences between years can also be responsible for the observed variable expressivity considering that the FGTH population was analyzed for mode of reproduction in 2005 and the SGTH population in 2007. Although Burton (1982) showed that the environment has little or no effect on the expression of apomixis in bahiagrass, seasonal variation of apospory was reported for *Paspalum cromiorrhizon* Trin. (Quarin 1986). Further research would be needed to identify these additional genetic factors that contribute to the expression of apomixis in bahiagrass.

The most successful forages for the southeastern USA have a prostrate growth habit that allows them to maintain their growing points without being removed by grazing animals. When diploid bahiagrass germplasm was subjected to multiple cycles of recurrent phenotypic selection for higher forage yield, populations with a more upright growth habit and lower tolerance to the stress caused by grazing were obtained (Gates et al. 2004). Thus, growth habit needs to be considered among the most important characteristics when breeding this species. Marked variability for spreading ability and plant height was observed in the SGTH population (Table 7). A high

proportion of this variability for growth habit was estimated, using broad-sense heritability estimates, to be the result of genetic variation among the progeny. In addition, the proportion of heterotic hybrids was also high for spreading and plant height (Table 6). These results indicate that selection can be efficiently used to develop clones with desirable growth habit. Previously reported genetic variance estimates and proportion of heterotic hybrids for the FGTH population (Acuña et al. 2009) were similar to the average estimates now reported for the SGTH (Table 7). This is another indication that large genetic variation for growth habit was contained in the SGTH population, and that one cycle of selection has not reduced this variability. These findings also indicate that a large variability for growth habit is present in the tetraploid bahiagrass germplasm.

An advantage of bahiagrass for use as a forage and utility turf is that it is easily established with seed. Production of inflorescences is one trait that can be recorded early in the evaluation of a large segregating population as an indirect estimation of seed production. The variation observed for production of inflorescences in the SGTH population was lower than that reported for the FGTH (Table 7). In addition, a large portion of the variation observed for the SGTH was attributed to environmental differences. The decline in variance and heritability estimates indicates that one cycle of selection significantly reduced the genetic variability for this trait. Moreover, the proportion of hybrids exhibiting heterosis declined by the second cycle of hybridization (Table 7).

Warm-season grasses have a delimited growing season in subtropical areas mainly because of photoperiod responses (Sinclair et al. 2001). This physiological response, that seems to be a mechanism of freeze damage avoidance, reduces forage production during spring and fall. Efforts are being made to reduce this photoperiod sensitivity and increase freeze resistance by genetic manipulation of bahiagrass at the diploid and tetraploid level (Blount and Acuña 2009). A large variation was observed among the SGTH for cool-season regrowth and freeze resistance (Table 7). A large part of this variation was attributed to genetic variation based on the heritability estimates. A minimal reduction of genetic variance and heritability estimates for spring and fall regrowth was observed when comparing estimates for

Table 7 Genetic variance and broad sense heritability (H^2) estimates for several agronomic characteristics, and proportion of heterotic hybrids (PHH) for the first generation of tetraploid hybrids (FGTH) created in 2004 (Acuña et al. 2009), and the second generation of tetraploid hybrids (SGTH) created in 2006

Trait	FGTH			SGTH		
	Variance	H^2	PHH	Variance	H^2	PHH
Spreading	102.9	0.69	36	86.8	0.80	15
Height	55.5	0.82	50	68.2	0.79	48
Inflorescences	495.8	0.88	31	147.3	0.48	8
Spring regrowth	0.88	0.82	47	0.73	0.64	29
Fall regrowth	0.82	0.85	32	0.61	0.63	38
Freeze resistance	0.25	0.65	38	0.58	0.77	32

the FGTH and SGTH populations (Table 7). This small change reflects the effect of one cycle of phenotypic selection on the genetic variability for cool-season regrowth. In contrast, the genetic variance for freeze resistance was larger for the SGTH compared with that for the FGTH. These results might relate to the fact that the SGTH population was exposed to lower temperatures before the data were collected. While the FGTH population was exposed to a minimal temperature of -2°C , the SGTH population was exposed to -6°C before freeze resistance was estimated. The genetic variability for the observed freeze resistance in the FGTH and the SGTH population did not change appreciably indicating the possibility to continue with subsequent cycles of hybridization and selection.

In conclusion, hybridization between sexual and apomictic bahiagrass clones can be used efficiently to generate a large segregating population. Minimal reduction of the genetic variability for traits of agronomic interest can be expected after one cycle of selection. A considerable high proportion of hybrids exhibiting heterosis for agronomically important traits can be expected when using this breeding approach. The low number of highly apomictic genotypes that can be found in each generation is a major limitation of this technique. Variation of apomixis expressivity can also be expected among generations.

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