Breeding Tetraploid Bahiagrass for Traits Conducive to Sod-based Crop Rotations

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INTRODUCTION

Bahiagrass, *Paspalum notatum* Flügge, is currently one of the most important pasture and utility turf species in Florida and the southern Coastal Plain region of the USA (Gates et al., 2004). It has been successfully utilized in sod-based crop rotations as a means of supporting livestock grazing and improving soil properties for successive crops (Katsvairo et al., 2006; Wright et al., 2004). Additionally, seed-propagated bahiagrass is a more economically viable alternative to vegetatively propagated sod in short (2-year) rotations.

Bahiagrass is a polymorphic species that contains races with different ploidy levels and linked reproductive characteristics. While diploids reproduce sexually (Burton, 1955), tetraploids reproduce asexually via apomixis (Burton, 1948). Apomixis is an asexual mode of reproduction where megasporogenesis and fertilization of the egg by a male gamete are bypassed, resulting in the production of clonal progeny. Apomixis therefore allows perpetuation of a fixed genotype, including complex, high yielding hybrids. This technique may provide a tremendous advantage in plant breeding and seed production (Grimanelli et al., 2001). All bahiagrass tetraploid cultivars released in the USA have been superior apomictic ecotypes selected from introduced germplasm, such as the cultivars 'Argentine' (PI 148996), 'Paraguay 22' (PI 158822), and 'Wilmington' (PI 434189) (Gates et al., 2004). However, a segregating population can be generated for breeding purposes or for genetic analyses by making crosses between sexual induced tetraploid plants and apomictic tetraploid plants.

Genotypic variability expresses itself in a myriad of traits; yield being the most notable. Traits favored in a bahiagrass breeding program may include cold tolerance, disease tolerance, forage quality, and nutrient utilization. Nutrient interception and uptake by plant roots, as in the case of nitrates, ensures that fertilizers applied to the soil are being removed effectively, resulting in potentially less run-off and leaching losses.

Nitrogen management in Florida is particularly important since nitrates can rapidly leach through the sandy soils and Karst geology of upper Florida, thereby impacting groundwater and spring ecology. High root densities, such as those associated with perennial forages, can remove over 80% of applied nitrogen. In comparison, row crops may remove less than 60% of applied nitrogen. A more effective root system can be particularly important for production systems located on the sandy southeast Coastal Plain soils since high rainfall events move nitrates rapidly from surface soil into the subsoil. Bahiagrass is a good candidate for N removal since it has nutrient storage capability in its stolons (Blue, 1972; Rodriguez et al., 1973) and posses a large, deep fibrous root system (Ball et al., 2002). Nitrogen removal can be increased by selecting cultivars with greater biomass production and/or greater tissue N content. Unlike yield, it is not well known how much genotypic variability exists among bahiagrass cultivars in terms of N capture and removal in low or high N input systems. Muchovej and Mullahey (2000) found approximately a 25% difference in seasonal yield and a 10% difference in seasonal forage N content among 5 commercially available cultivars grown under low N (50 lb/A/yr). It is possible that cultivar differences may be greater under higher N inputs. Since bahiagrass N content tends to be relatively low in comparison to other C_4 grasses (i.e. bermudagrass and limpograss), moderate increases in bahiagrass forage N accumulation might improve overall forage quality without reaching a point of excessive tissue nitrates.

Other traits, such as nematode resistance is especially beneficial when using bahiagrass in a crop rotation. Some of the positive effects associated with a bahiagrass rotation, such as yield increases by crops following the sod, have been attributed to nematode and disease suppression (Gates et al., 2004). The genotypic response to nematode pressure is only beginning to be evaluated in our bahiagrass research program.

The objectives of this research were 1) to characterize the bahiagrass tetraploid germplasm, 2) to generate a segregating population by hybridizing sexual and apomictic tetraploid genotypes, and 3) to initiate screening of selected apomictic progeny for forage production, nitrogen accumulation, and nematode resistance.

MATERIALS AND METHODS

Germplasm Characterization

Twenty of the most vigorous plants from a population of 300 artificially induced tetraploids (Quesenberry and Smith, 2003) were grown in the field and greenhouse from summer 2004 to the end of summer 2005. Two sexual tetraploid lines, Q4188 and Q4205 (Quarin et al., 2003), the cultivars Argentine (PI 148996) and Wilmington (PI 434189), and the experimental hybrid Tifton-7 (Burton, 1992) were also grown in a greenhouse and field during this time period. The ploidy level of these genotypes was confirmed by flow cytometry, and their mode of reproduction was determined by embryo sac observations as described by Acuna, 2006. A segregating population containing 600 hybrids was generated by crossing the best 6 sexual plants as female parents and Argentine and Tifton 7 as male parents.

Progeny Evaluation

Entire progeny (Phase 1)

The progeny obtained by hybridization of sexual and apomictic genotypes were transplanted into the field in May 2005. Initial assessments were based on plant diameter, plant height, recovery (regrowth) from clipping in fall and spring and frost resistance. Plant diameter was estimated using the average between the longest and the shortest diameters of a given plant. Plant height was measured from the base of the plant to the top of the standing canopy. Starting on 20 July (50 days after transplanting or DAT), plant diameter and height were taken at 4 wk intervals. On 21 September all plants were defoliated to approximately 3 inches above the soil level. Regrowth was visually rated on 28 October, and 24 May of the following growing season using a 1 to 5 scale, where 1 equaled plants with the lowest amount of forage and 5 equaled plants with the greatest amount of forage. Frost resistance was visually estimated on 28 December after two consecutive frost events on 23 and 24 December, with temperatures of 28 and 30 °F respectively, using a 1 to 5 scale, where 1 equaled the least frost resistant, and 5 equaled the most frost resistant plant. In an attempt to estimate the segregation for apomixis and to collect seed before the field evaluations were completed, a group of 71 plants was selected on 19 August, 2005. These plants were classified as sexual, apomictic, and facultative apomictic based on mature embryo sac observations.

Selected apomictic hybrids (Phase 2)

Small plots (65 ft^2) with 7 of the most promising apomictic hybrids were established in Gainesville in 2006. Two other apomictic hybrids, and cultivars Argentine and the experimental hybrid Tifton-7 were also included. These plots were fertilized at the beginning of the growing season with 16-4-8 at 445 lb/A. Plots were harvested on October 31 and forage mass and N concentration were determined. Plots were sampled again in April 2007 for forage and stolon/root mass.

RESULTS AND DISCUSSION

Germplasm Characterization

Cultivars

Flow cytometric analysis confirmed that the cultivars Argentine, Wilmington, and the experimental hybrid Tifton-7 were all tetraploid. Embryo sac observations indicated that the cultivars Argentine and Wilmington were highly apomictic plants. Ninety five percent of the analyzed ovules had multiple apomictic sacs for both cultivars; while the other 5% had multiple apomictic sacs in addition to one meiotic sac. The experimental hybrid, known as Tifton-7, was generated throughout a long and intricate breeding approach (Burton, 1992). Although this hybrid has been shown to be significantly more productive than Argentine, it has remained as an experimental hybrid due to concerns regarding seed production. It was also highly apomictic with 95% of its ovules containing only apomictic embryo sacs, and 5% containing both apomictic and meiotic sacs sharing the same ovule. Although Burton (1992) stated that Tifton-7 was significantly more fertile than Argentine, our results showed no significant differences in self- and cross-fertility between these two genotypes. They produced 31% of seed when self-pollinated and 36% when open-pollinated. Thus, the previously reported differences in seed production might be related to number of inflorescences per unit area.

Induced tetraploids

Flow cytometric analysis confirmed that the 20 genotypes from the artificially induced population were all tetraploid. In addition, all of them were determined by mature embryo sac observations to reproduce sexually. Although large variability in terms of sexual expression was recorded for naturally occurring tetraploids (Martínez et al., 2001), highly sexual plants have not been found in natural populations.

In addition to being the most vigorous plants in a large population these 20 genotypes were reasonably cross-fertile, and self-sterile. They produce 2 % of seed when self-pollinated and 22 % of seed when cross-pollinated. Thus, results indicated that these 20 sexual tetraploids should be considered as good female counterparts for breeding tetraploid bahiagrass.

Progeny Evaluation

Entire progeny

Plant diameter and plant height were measured in order to characterize the growth habit of the progeny. Progeny showed significant differences for both variables during the entire growing season indicating a marked variability in terms of growth habit. On 20 July, 50 days after transplanting, progeny were ranked based on their diameter. A range from 5 to 11 inches in diameter was observed for the first measurement. The progeny showed an additional spread of between 2 to 6 inches after an additional month and between 4 to 9 inches two months later. The rate of spread between the first and the second measurements was higher than between the second and the third measurements. This difference could be related with the daylength for each period. The average daylength between the first and the second measurements (13 h 30 min) was 50 min longer than the average daylength between the second and the third measurements (12 h 40 min).

Plant height for a given progeny did not vary significantly among the three measurement periods, indicating that the progeny did not increase in height from 20 July until the end of the growing season. These results indicate that increases in canopy height occurred early and reached maximum height soon after transplanting. Some progeny showed a marked vertical or upright growth habit while other progeny showed a marked horizontal or prostrate growth. The most successful forage grasses for Florida pastures have tended to have exhibited marked prostrate growth resulting in persistence under grazing, and they have the capability to spread and colonize new areas quickly. For example, our Fl-4-36-1 x Argentine, Fl-2-2-7 x Tifton-7, Fl-71 x Argentine, and Fl-106 x Tifton-7 hybrids may potentially be good choices for pastures since they have a more prostrate growth habit.

The progeny were determined to be significantly different in terms of plant regrowth during fall 2005 and spring 2006. The top four populations, Fl-4-36-1 x Argentine, Fl-106 x Tifton-7, Fl-2-2-7 x Tifton-7, and Fl-71 x Argentine were determined to be the most suitable combinations for extending the growing season. Interestingly, the four populations that spread fastest between 1 June and 21 September showed better plant regrowth either before or after the winter. Additionally, these four populations had a better general vegetative vigor throughout the initial growing season.

Large variability was also observed among the progeny in terms of frost resistance. The four progeny that showed superior vegetative vigor also were among the most frost resistant, populations. Among the best performers in this category was Fl-106 x Tifton-7 progeny.

To gain time and realize genetic advance in the evaluation process, a group of 71 plants was phenotypically selected on 19 August 2005. Both inflorescences at anthesis and seed were collected from all plants. The plants were classified as sexual, apomictic, and facultative apomictic based on embryo sac observations. Eight of them were classified as apomictic because more than 90 % of their ovules contained apomictic embryo sacs. Twelve plants were classified as facultative apomictic because a low percentage (less than 40 %) of their ovules showed apomictic embryo sacs while the rest of their ovules showed sexual embryo sacs. The remaining 51 plants were classified as highly sexual because all of their ovules contained only a single sexual embryo sac. By grouping both apomictic and facultative apomictic plants, it is estimated that 28 % of the plants from this sample inherited the gene(s) for apomixis.

Selected apomictic hybrids

High variability was observed for forage mass among these hybrids. The average forage mass was 3110 lb/A varying from 1550 lb/A to 4570 lb/A (Fig. 1). Cultivar Argentine produced 2130 lb/A while Tifton-7 produced 3210 lb/A. For comparison, Tifton-7 yields in our trials have been comparable to the diploid, Tifton-9 yields. Five novel hybrids (3 from Argentina and 2 from the UF program) produced more forage mass than Tifton-7. However, these results are preliminary and further evaluation is required. An initial spring 2007 sampling of stolons + roots resulted in a 3-fold difference in stolon/root dry mass among hybrids (Fig. 2). In general, there appears to be a moderate correlation between above-ground and stolon/root biomass ($r^2 > 0.50$). Even so, enough variation among hybrids may exist to use stolon/root mass as a selection criterion for specific forage systems. Additional measurements in 2007 and 2008 will determine if this is the case.

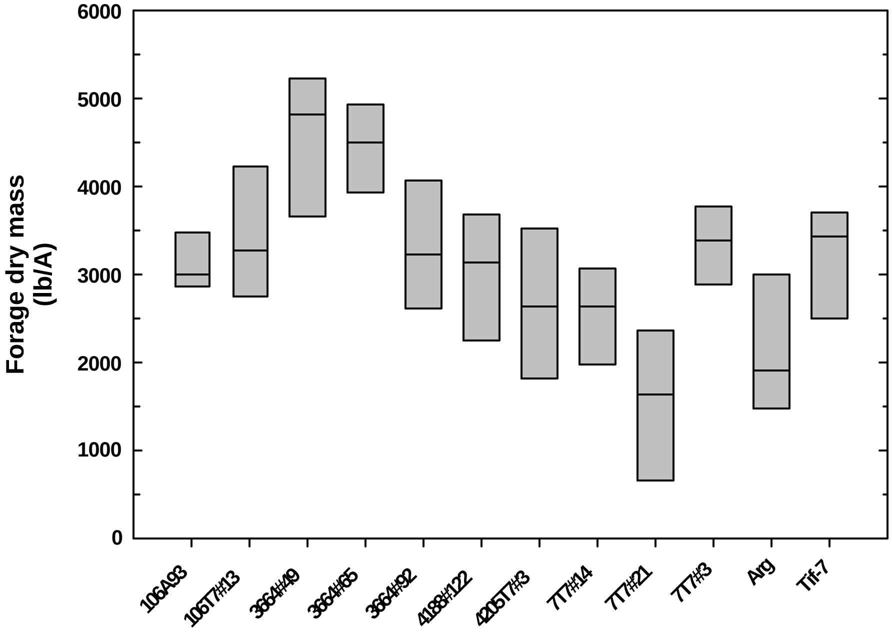

Fig. 1. Box plot of fall 2006 forage field harvest of selected apomictic hybrids compared with Argentine (Arg) and Tifton-7 (Tif-7). Bar length equates to value range and median is represented by horizontal line (n = 4).

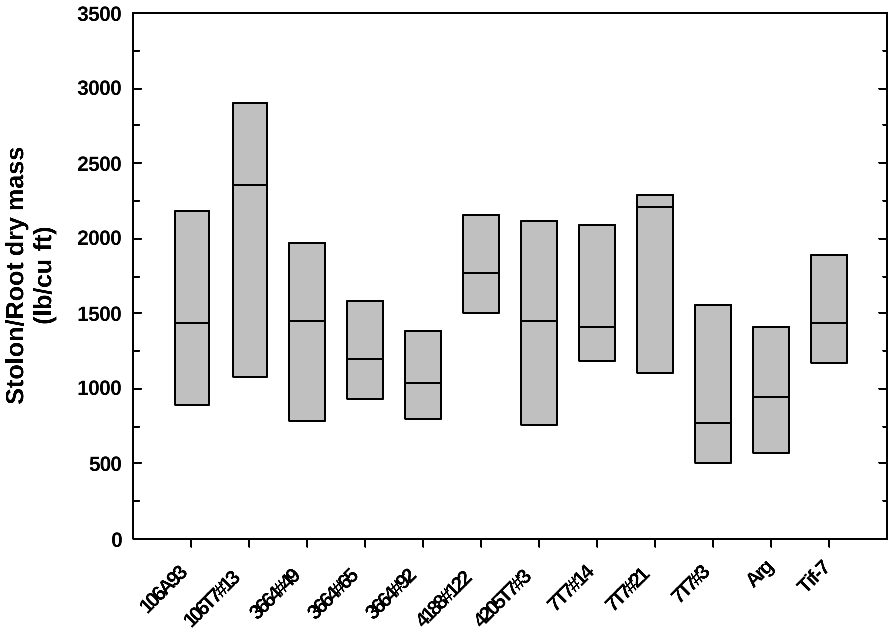

Fig. 2. Box plot of spring 2007, stolon + root harvest from selected apomictic hybrids as compared with Argentine (Arg) and Tifton-7 (Tif-7). Each 16 cu inch soil core was taken below an established plant. Bar length equates to value range and median is represented by horizontal line (n = 4).

Some hybrid variability was observed for leaf nitrogen concentration in the fall sampling. The average nitrogen concentration varied from 0.9 to 1.2 % (Fig. 3). The generally low nitrogen concentration in all the harvested forages was likely related to its maturity (first and only cutting) at the time it was collected (Fall, 2006). Based on the preliminary data, variability observed with nitrogen accumulation was primarily defined by genotypic differences on forage production but not in all cases. For example, hybrid 106T7#13 removed as much N as the highest yielding hybrids containing lower tissue N (Fig. 4). Total N removal by biomass ranged from 21 lb/A to 54 lb/A with an overall average of 37 lb/A. Sampling will continue in 2007 to determine cultivar variability in N content and removal over an entire growing season using a low and high N application rate.

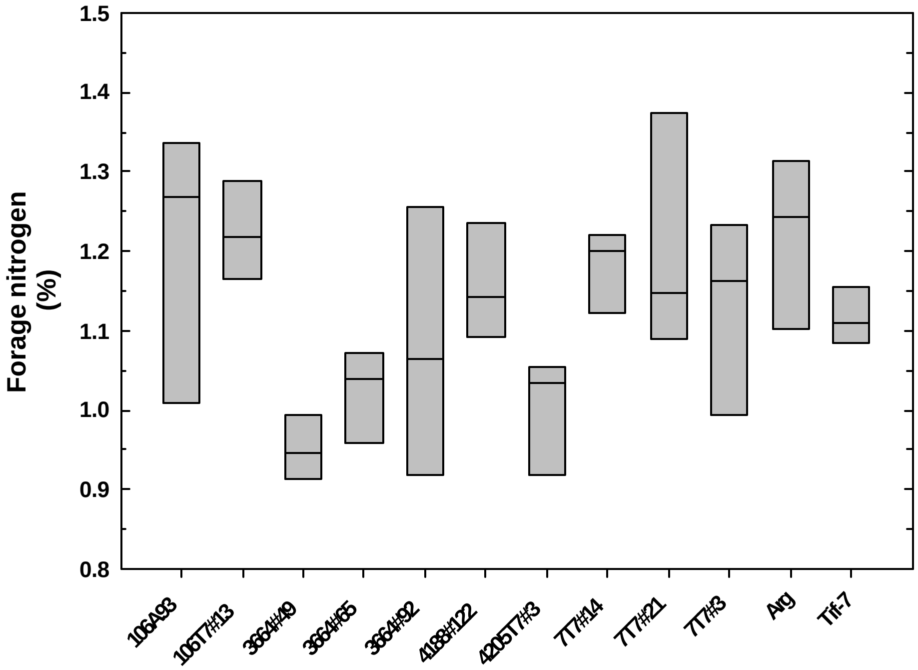

Fig. 3. Box plot of fall 2006 forage nitrogen content of selected apomictic hybrids compared with Argentine (Arg) and Tifton-7 (Tif-7). Bar length equates to value range and median is represented by horizontal line (n = 4).

Although still in its initial testing phase, it becomes clear that given enough genotypic variability among progeny, customized bahiagrass cultivars may be used in specific systems. For example, one bahiagrass cultivar might be most appropriate for a hay system, another for a pasture system and then another for a sod-based crop rotation. Table 1 provides examples of how system attributes may differ and which of our tetraploid evaluations might fit into the various systems. Hay production would require good yield and stolon/roots to protect against drought or other stresses. Good N recovery means less fertilizer inputs. In a pasture system, greater crude protein, particularly under low N input (typical of many pastures in Florida) and greater stolon/root mass would be desirable. Greater forage yield may not be as important, particularly since summer production can get ahead of grazing pressure with many of the currently available cultivars. Sod-based rotation requirements may be similar to pasture requirements, with the exception that a lower stolon/root producer may be easier to manage when it comes to rotating in with the succeeding row crop.

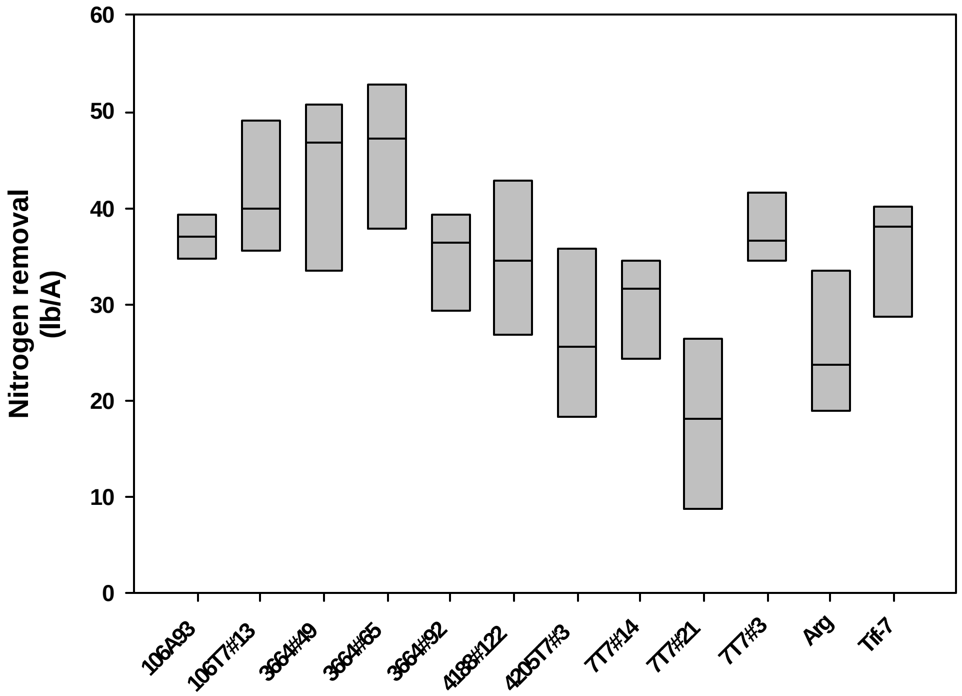

Fig. 4. Box plot of fall 2006 forage nitrogen removal in biomass of selected apomictic hybrids compared with Argentine (Arg) and Tifton-7 (Tif-7). Bar length equates to value range and median is represented by horizontal line (n = 4).

Table 1. Attributes and bahiagrass genotypes conducive to sustainable agricultural systems.

System	Desirable Attributes	Potential Tetraploids
Hay Production	greater yield greater stolon and root mass greater N recovery	106T7 #13 3664 #49
Pasture	greater crude protein greater stolon and root mass	Argentine Tifton-7 106 x A #93 7 x T7 #21 106 T7 #13
Sod-based Crop Rotations	greater crude protein (grazing) greater N recovery less stolon and root mass	3664 #92 7T7 #3

Nematode resistance/tolerance was not listed under the Table 1 attributes but it is a trait that is highly desirable for use in a sod-based crop rotation. Greenhouse screening for root-knot nematode resistance or susceptibility was initiated in spring, 2007, using novel tetraploid hybrids and commercially available tetraploid and diploid cultivars. Data collection will begin in early summer, 2007. Chances are good that some genotypic variability exists among bahiagrass cultivars since variability has been observed for many other traits. For example, genotypic variability exists for biomass and N content (Muchovej and Mullahey, 2000) and even herbicide tolerance (Bunnell et al., 2003). Additionally, large genotypic variability to root-knot nematode resistance was found in Italian and perennial ryegrasses (York and Cook, 1988).

Conclusions

Bahiagrass tetraploid hybrids were successfully created by crossing the best sexual and apomictic genotypes, resulting in a ratio of 3 sexual to 1 apomictic hybrids. Screening was initiated in the field and greenhouse to evaluate the new genotypes for forage production, nitrogen utilization and nematode resistance. There were clear genotypic differences for forage production and N removal, where total N removal was primarily dictated by forage yield under low N inputs (50 lb/A). Other traits, such as stolon/root mass and forage N concentration were not as highly variable but additional sampling under greater N application rates need further testing. Work will also continue to determine N uptake with soil depth and nematode resistance of the different promising genotypes.

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