Breeding Apomictic Forage Grasses: Molecular Strategies

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INTRODUCTION

The breeding and improvement of perennial forage grasses can range from a straight forward process whereby remarkable progress is made in the development of improved germplasm to a challenging and frustrating undertaking in which minimal or no progress is made. Success comes more readily in grasses that are meiotically stable, cross-pollinated, and sexual. However, at the opposite end of the spectrum are species that are meiotically irregular, complex polyploids and reproduce by apomixis. Unfortunately, many warm-season perennial forage grasses are polyploid apomicts and because of this most, forage improvement programs in the southern United States are confronted with these problems.

BREEDING HISTORY

The Texas Agricultural Experiment Station and USDA-ARS cooperative forage improvement program at College Station, TX has a long and successful history of addressing these complex problems, especially apomixis. The emphasis of this cooperative state/federal program has been to learn more about apomixis and to develop novel approaches to breed apomictic grasses. Under the leadership and direction of Dr. E.C. Bashaw, this program obtained national and international recognition as the premiere apomictic grass breeding program in the world. Research findings from this program demonstrated that 1) apomixis is genetically controlled (Taliaferro and Bashaw, 1966), 2) apomixis could be used as a dynamic breeding tool when sexual germplasm is available (Taliaferro and Bashaw, 1966; Bashaw and Funk, 1987), and 3) novel breeding methodologies, such as the fertilization of unreduced egg cells, could be used to create new apomictic genotypes in obligate apomicts (Bashaw, et al., 1992; Hussey, et al., 1993). These findings were used in the development and release of several new apomictic forage grass cultivars (Bashaw, 1968; Bashaw 1980). With this legacy, the program continues to address apomixis and other fundamental problems that negatively impact or prevent the breeding of warm-season grasses. Besides elucidating these basic reproductive problems, another major objective of the program is to use the findings from these studies to breed and release improved forage grass cultivars for the southern United States. This cooperative research program currently is working primarily with buffelgrass (*Pennisetum ciliare* [L.] Link syn *Cenchrus ciliaris* L.), dallisgrass (*Paspalum dilatatum* Poir.) and kleingrass (*Panicum coloratum* L.). Of the three species, more attention is currently being given to buffelgrass.

Buffelgrass is a warm-season, perennial bunch grass that is used as a forage grass through out the semi-arid tropics. The grass has excellent drought tolerance and produces large quantities

of high quality forage with minimal moisture. Another strength of the species is its ease of establishment. Unfortunately, it lacks winter hardiness, is adapted to only a narrow range of soil types, and is susceptible to several diseases. The most common chromosome number reported for buffelgrass is 2n=4x=36; however, other numbers have been reported. Based on meiotic chromosome pairing behavior, the 36-chromosome cytotypes have been classified as segmental allotetraploids. It is an apomictic species; however, a few rare sexual plants have been discovered. These rare sexual plants have made it possible to improve the species by hybridization. The sexual plant is used as the female parent and is pollinated with pollen from an apomict. The F_1 hybrids are phenotypically different from either parent and segregate for method of reproduction. In addition to the development and release of new apomictic cultivars, this breeding approach also provides the opportunity to use molecular tools in the breeding and improvement of buffelgrass.

GENETIC MAPPING

Currently we are developing molecular markers to use as a tool in breeding buffelgrass. This species was chosen because 1) its an economically important forage grass, 2) it has a relatively small genome size (ca. 3200 MB) for a polyploid species, 3) large populations segregating for multiple traits (i.e. apomixis, rhizomes, inflorescence type, etc.) already exist, 4) apomixis and sexual reproduction have been studied in this species, and 5) it is in the tertiary gene pool of pearl millet, an important grain crop.

The initial objective of this program is to develop low density genetic maps of buffelgrass using both homologous (cDNA) and heterologous (cDNA and gDNA) probes. Because of the polyploid nature of buffelgrass, markers are scored and mapped as single dose restriction fragments resulting in separate male and female maps (Wu et al., 1992). The current buffelgrass genetic maps consist of 42 and 47 linkage groups for the male and female maps, respectively (Jessup et al., 2000). Additionally, the use of heterologous probes and the simultaneous placement of a subset of the buffelgrass probes on a sorghum genetic map, provide an opportunity for detailed comparisons of genomic regions of forage grasses such as buffelgrass with grain crops (Bowers et al., 2000; Paterson et al., 2000). At this time, we are continuing our efforts to improve marker density in buffelgrass using a combination of markers.

The low density genetic map will then serve as a platform to initiate a QTL mapping proaject in buffelgrass. Limited work has been conducted using QTLs in forage species. Exceptions to this include rhizome development in johnsongrass, QTLs for yield, IVDMD, ADF, etc. in maize harvested for silage, and drought resistance in *Lolium-Festuca* hybrids. Initial QTL mapping efforts in buffelgrass will utilize an F_1 hybrid population consisting of ca. 200 plants. The mapping population was established in the field during the summer of 1999 and is segregating for numerous traits (i.e. method of reproduction, plant height, +/-rhizomes, etc.). During 2000, this population will be scored for the traits listed in Table 1 and QTL mapping initiated in 2001.

In addition to our broader genetic mapping efforts, we are also interested in obtaining a better understanding of the genetic control of apomixis in perennial grasses. Numerous models have been suggested for the genetic control of apomixis in plants (Asker & Jerlilng, 1992; Carman, 1997; Moggie, 1992; Savidan, 1983; Sherwood, 1994; Taliaferro & Bashaw, 1966) yet the precise genetic mechanism regulating its control is unknown. There are several possible explanation for the inability to understand the genetic control of apomictic reproduction in plants including the 1) use of inappropriate methods for determining apomixis (seed-set), 2) the use of untested genetic models without supporting genetic (marker) or cytogenetics data, 3) failure to explain partial (facultative) apomixis, etc. Nonetheless, it is evident that the application of molecular tools coupled with a good developmental understanding of plant reproductive biology will lead to our ultimate understanding of this elusive trait.

Both RFLP and AFLP based studies in several forage grasses have identified markers that are tightly linked to apomixis. In our research, we have also identified ca. 10 homologous and heterologous probes (RFLP) linked to apomixis in addition to ca. 30 markers which were identified using bulked segregant analysis and selective restriction fragment amplification (AFLP). It is our intent to further saturate this region to 1) determine preferential chromosomal pairing relationships between the region containing the gene(s) controlling apomixis and other regions of the buffelgrass genome, 2) to study similar chromosomal regions in sexual buffelgrass genotypes and other sexual taxa, and 3) to ultimately move toward cloning the gene(s) controlling apomixis.

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Table 1. Traits currently being evaluated for QTL mapping in Buffelgrass.

Forage production	Flowering time
Forage composition	Seed-set
Plant height	Plant color
Rhizomes	Apomixis
Tillering	Seed Production