Interspecific Hybridization Between Pembagrass (*Stenotaphrum dimidiatum)* and St. Augustinegrass (*S. secundatum*) Using Embryo Rescue

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Abstract -

St. Augustinegrass is a popular turfgrass grown in the southern part of the United States with the best shade tolerance of the warm-season grasses. Its stoloniferous growth habit lends itself to vegetative propagation by turfgrass producers. Ploidy differences exist between popular cultivars where diploids such as 'Raleigh' and 'DelMar' have 2n=2x=18 chromosomes and polyploids such as 'Floratam' have 2n=3x=27. Polyploids have been characterized as having genes for chinch bug resistance, but have been under utilized as a breeding resource due to sterility resulting from ploidy differences. At the ASA meetings in 2004 we described the use of embryo rescue as a tool that enabled the recovery of viable progeny from polyploid/diploid crosses. Sterility barriers exist which also limits the use of pembagrass as a genetic resource in crosses with St. Augustinegrass. Certain pembagrass genotypes have been characterized as having genes for resistance to gray leaf spot, southern chinch bug and sting nematode and may prove to be a valuable resource for St. Augustinegrass improvement. This year we report the expanded use of embryo rescue technology to enable interspecific hybridization between St. Augustinegrass and pembagrass.

Introduction –

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is widely used as a turfgrass in warm humid, tropical and subtropical climates. Propagation is usually vegetative, by either stolon cuttings, plugs, or sod. Six other species of the *Stenotaphrum* genus are endemic to the Old World and are also confined mainly to shorelines from Africa to the South Pacific (Sauer, 1972). Morphologically, pembagrass [*S. dimidiatum* (L.) Brongn.] is the species most similar to St. Augustinegrass; the two species are separated primarily by number of spikelets per raceme, leaf pubescence and chromosome number.

Various chromosome counts are found in St. Augustinegrass with diploids having 2n=2x=18 chromosomes, triploids (2n=3x=27) and tetraploids (2n=4x=36) (Long and Bashaw, 1961). Similarly, various chromosome counts have been found for *S. dimidiatum,* 2n=36 from Sri Lanka (Gould and Soderstrom, 1974), 2n=48 from Malagasy Republic (Sauer, 1972) and 2n≈60 from Mauritius (Busey et al., 1993).

Resistance to several pests of St. Augustinegrass are found in pembagrass. *S. dimidiatum*, PI-365031 has resistance to gray leaf spot disease caused by *Pyricularia grisea*(Cke.) Sacc. (Atilano and Busey, 1983) and the southern chinch bug (Reinert et al., 1986; Busey, 1990); *S. dimidiatum,* FL-2195 has resistance to the sting nematode (Busey et al., 1993). *S. dimidiatum* is a good first candidate for use in interspecific crosses. St. Augustinegrass is easy to manipulate in breeding (Philley et al., 1993). Inflorescence initiation is photoperiod-controlled (Dudeck, 1974). Spikelets are relatively large and easy to emasculate (Philley, 1994). Sterility barriers prevent the production of fertile seed when *S. dimidiatum* is used as a female and *S. secundatum* as the male.

Embryo culture or rescue is a valuable *in vitro* tool for breeding (Bridgen, 1994). It has been used effectively to rescue embryos from interspecific and intergeneric crosses where the pollination event leads to zygote formation but the embryo fails to complete its development resulting in abortion. This technique has aided in broadening the genetic base by introducing genes which enhance productivity by virtue of their enhanced resistance to biotic and abiotic stresses. The approach has been used effectively in rice (Brar, 1997), Brassica (Inomatu, 1993), eggplant (Kashyap et al., 2003), and wheat (Bai et al., 1994) to name a few.

Last year (ASA Annual Meeting in Seattle, 2004) we reported the successful recovery of over 200 progeny from crosses with various sterile St. Augustinegrass polyploidy females by means of embryo rescue techniques (Genovesi et al., 2004). Using similar embryo rescue techniques, we report the successful recovery of approximately 130 presumptive interspecific progeny resulting from the cross of *S. dimidiatum* x *S. secundatum.*

Material and Methods -

Germplasm – Diploid (2n=18) commercial St. Augustinegrass cultivars were obtained in order to be utilized as the pollen parent in crosses with female pembagrass, *S. dimidiatum*. The diploid *S. secundatum* lines included: 'Raleigh', 'DelMar', 'Nortam' and 'TAES2949'. Only two cultivars of *S. dimidiatum* were available from the Plant Genetic Resources Conservation Unit, USDA, Griffin, Georgia. They are PI 289729 from the Malagasy Republic and PI 365031 from the Republic of South Africa.

Breeding Methods – Plant breeding methods described by Philley et al., 1993 were utilized in making pollinations. A camel hair artist's brush was used to apply pollen to the stigma of the emasculated pembagrass female. Spikelets came into flower from the tip of the inflorescent to base over the period of a week with newly exerted flowers and stigmas treated daily as indicated above. Further pollinations ceased after a week at which time the flower/test tube was allowed to remain under the florescent lights for 2 weeks following the last pollination.

Embryo Rescue – Flowers with spikelets pollinated 2 to 3 weeks prior, were taken to the laminar flow hood for aseptic isolation of the developing embryo. The inflorescence was removed from the peduncle and placed in a petri dish containing enough 70% ethanol (EtOH) to cover the bottom. With the aid of a dissecting microscope and forceps, spikelets were opened to reveal their contents. The ovule was placed on a sterile petri dish lid and left in the flow hood with a bead of 70% EtOH surrounding it and allowed to air dry for approximately 10 min. The ovule was aseptically opened with forceps and the developing embryo removed and place on solidified nutrient medium. The medium was either a half strength MS (Murashige and Skoog, 1962) or McCown (Lloyd and McCown, 1981) basal medium with 4 – 5% sucrose as a carbon source and osmoticum. Cultured embryos were placed under an 18 photoperiod at 25ºC. After 2 - 3 weeks individual plants were moved to a 2..54 cm diameter test tube with the same medium for another 3 – 4 weeks at which time they were ready to plant. Plants were taken to the greenhouse where they were potted in a soil mix and placed on a misting bench to harden off for a week before being moved off.

Flow Cytometry - A modified technique of that reported by Huff and Palazzo, 1998 was used to determine the DNA content in interspecific progeny and parents.

References –

Atilano, R. A. and Busey, P. 1983. Plant Disease 67: 782-783.
Bai, D. et al. 1994. Genome 37(3):410-418.
Brar, D. S. and G. S. Khush. 1997. Plant Molecular Biology 35: 35-47.
Bridgen, M.P. 1994. HortScience 29(11): 1243 – 1246.
Busey, P. 1990. Crop Sci. 30: 588-593.
Busey, P. et al. 1993. Crop Sci. 33: 1066-1070.
de Wet, J. M. J. 1979. Origins of polyploids. P. 3-15. *In* W. H. Lewis (ed.) Polyploidy: Biological relevance. Plenum Press. New York.
Dudeck, A. E. 1974. Flowering in several selections of St. Augustinegrass. In: (eds) E. C. Roberts, Second Int. Turfgrass Res. Conf. Proc., Blacksburg, VA, 15-21 June 1973. ASA and CSSA, Madison, WI. pp. 74-78.
Genovesi, A. D. et al. 2004. ASA Annual Meeting, Seattle, WA. Abstract # 4290.
Gould, F. W. and T. R. Soderstrom. 1974. Can. J. Bot. 52: 1075-1090.
Huff, D.R. and A. J. Palazzo. 1998. Crop Sci. 38: 445-450.
Inoumatu N. 1993. Monographs on TAG 19: 94-107.
Kashyap, V. et al. 2003. Scientia Horticulturae 97: 1-25.
Long, J. A. and E. C. Bashaw. 1961. Crop Sci. 1: 41-43.
Lloyd, G. and B. McCown. 1981. Proc. Int. Plant. Prop. Soc. 30: 421.
Murashige, T. and F. Skoog, 1962. Physiol. Plant. 15: 473- 497.
Philley, H. W. 1994. Inheritance of cold tolerance in St. Augustinegrass. M.S. thesis, Mississippi State Univ.
Philley, H. W. et al. 1993. Agronomy Abstracts, p. 163.
Reinert, J. A. et al. 1986. J. Econ. Entomol. 79: 1073-1075.
Sauer, J. D. 1972. Brittonia 24:202-222.

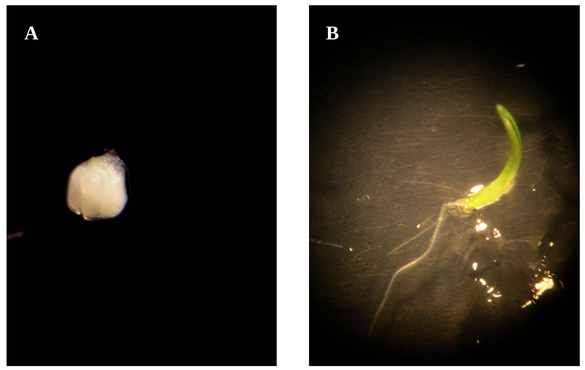

Figure 1 - A. Rescued PI 365031 x DelMar embryo 1 day after isolation with embryo axis facing up and a slightly attenuated scutellum (16x). B. Germinating plantlet resulting from embryo rescue 3 weeks after isolation (4x).

Table 1. Interspecific hybrid progeny derived from crosses between *Stenotaphrum dimidiatum* females and *Stenotaphrum secundatum* males in 2004/05 using embryo rescue.

Parents ♀ x ♂	Number Inflorescences	Number Spikelets	Number Progeny (%)
PI 289729 x Mercedes	2	56	0 (0)
PI 289729 x Nortam	1	24	1 (4.2)
PI 289729 x TAES2949	1	15	1 (6.7)
PI 365031 x DelMar	4	153	49 (32.0)
PI 365031 x Raleigh	1	60	19 (31.7)
PI 365031 x Nortam	1	35	12 (34.3)
PI 365031 x TAES2949	3	70	47 (67.1)
		Total	**129**

Results and Discussion –

A total of 129 progeny were produced from crosses with *S. dimidiatum* females using embryo rescue methodology (Table 1). Most of the progeny (127) resulted from crosses with PI 365031 while crosses with PI 289729 only returned 2 progeny. Flow cytometry was done on the progeny to determine the DNA content of cell nuclei. The two progeny resulting from crosses between PI 289729 (4.09 pg) and St. Augustinegrass [either Nortam (3.08 pg) or TAES2949(3.07 pg)] had intermediate nuclear DNA contents (3.91 pg and 3.80 pg respectively) consistent with interspecific hybridization. Crosses made with PI 365031 are somewhat more difficult to interpret (see Figure 2). Many of the presumptive hybrid progeny have nuclear DNA contents near that of the highest parent, PI 365031, or higher. Self pollination is unlikely since very little endosperm was present. This would suggest (1) that parthenogenetic development of the egg cell may be occurring some of the time or that (2) unreduced gamete/egg nuclei formation is occurring sometimes so that when pollinated, hybrid progeny with nuclear DNA contents exceeding either of the two parents are produced.

Conclusions -

- A total of 129 presumptive interspecific hybrid progeny were recovered using embryo rescue from pembagrass (*S. dimidiatum*) females using St. Augustinegrass (*S. secundatum*) males.
- Genetic background of the *S. dimidiatum* selection influenced the ease with which it crossed to *S. secundatum*. PI 365031 crossed more readily as a female than PI 289729.
- Flow cytometry showed DNA content of some progeny were intermediate to the two parents.
- In many cases, DNA content of the progeny clustered around the pembagrass parent or were even higher than the high parent. This may indicate that there is a high incidence of unreduced gamete formation resulting in higher levels of polyploidy (de Wet 1979) or parthenogenetic development of the egg is occurring.
- Flow cytometry can be used as a tool to characterize ploidy level of progeny resulting from a cross between parents with different ploidys.

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Figure 2. Histograms of DNA content in cell nuclei (pg / 2C) from four progeny populations resulting from crosses of the *Stenotaphrum dimidiatum* female, PI 365031, and St. Augustinegrass diploid males.