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Tissue culture-induced morphological somaclonal variation in St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze]

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With 2 figures and 1 table

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Abstract

Somaclonal variation has been observed in many plant species and is an alternative way to create variants and expand the germplasm pool. A large scale tissue culture experiment was conducted with St. Augustinegrass, an important turfgrass species for the southern USA, to induce somaclonal variation to enlarge the germplasm pool for breeding efforts. Using an improved protocol, approximately 7900 St. Augustinegrass plants were regenerated from cv. 'Raleigh', and 119 morphological variants were identified. Among the variants, 115 had a semi-dwarf growth habit with shorter and narrower leaves, and shortened internodes and stolons. However, 100 of them showed little vigour, which either grew very slowly or did not survive. The remaining 15 showed reasonable growth vigour and were further investigated in the field. Among them, 13 were semi-dwarf and 2 had longer leaves. In addition, 2 other variants, with variegated (yellow striping) leaves, or significantly thicker stems were also observed and characterized. The altered traits in the variant lines were stable during vegetative propagation and when grown in different environments.

Key words: semi-dwarf — somaclonal variation — St. Augustinegrass — tissue culture

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is a widely distributed coastal species in the tropics and subtropics, and an important turfgrass for lawns and sports fields. In Florida, 70% of lawns are estimated to be St. Augustinegrass (Busey 2003). St. Augustinegrass is a clonal crop and propagated by sprigging or sodding. Although most of the cultivars are diploid (2n = 18), some cultivars and germplasm are polyploid (2n = 27, 30, 32) including important cultivars such as 'Floratam' and 'FX10'. Although seeds are set within the ploidy levels, efforts to develop seeded commercial cultivars have not been successful (Busey 2003). St. Augustinegrass produces dense and attractive dark blue-green turf and is well adapted to most soils. A major drawback of St. Augustinegrass for lawns is its coarse texture. The goal of this research is to employ plant tissue culture to induce and characterize somaclonal variants which have finer leaf texture/semi-dwarf growth habit that can be used as germplasm for breeding purpose.

Somaclonal variation (SV) can be defined as genetically stable variation generated through plant tissue culture (Larkin and Scowcroft 1981), and is a breeding approach to create greater genetic diversity and expand germplasm pool for plant improvement and cultivar development. Jain (2001) reviewed the SV breeding practice and found that 22 cultivars had been released from SV with improved traits including yield, plant architecture, colour, pest resistance, and salt and heat tolerance. Cited examples included 'He Zu No. 8' wheat (*Triticum aestivum* L.) with high yield, 'Yidan No. 6' maize (*Zea mays* L.) with improved grain quality, 'CIMAP/bio-13' aromatic grass (*Cymbopogon winterianus* Jowitt) with increased oil yield, and 'DAMA' rice (*Oryza sativa* L.) with *Picularia* spp. resistance.

Induction and utilization of SV in St. Augustinegrass could create and enrich genetic diversity and be a promising approach for its improvement. However, no report has been published using SV in St. Augustinegrass breeding. Kuo and Smith (1993) initially cultured immature embryos of cv. 'Texas Common' on MS medium containing 1 mg/l 2, 4-D, followed by 4 weeks culture with 0.5 mg/l 2, 4-D plus 0.25 mg/l kinetin and found that approximately one third of the induced calli were able to regenerate into plantlets. An improved tissue culture technique using immature embryos cultured on MS medium containing 1 mg/l 2, 4-D and 0.5 mg/l BA (6-benzylaminopurine) led to enhancement in both callus induction and regeneration with rates near 98% and 48%, respectively (Li et al. 2006).

This manuscript reports a mass tissue culture efforts to regenerate approximately 7900 St. Augustinegrass plants from cv. 'Raleigh' *in vitro* to isolate somaclonal variants as an approach for breeding improvement of the turfgrass species. Visual selection of plants with altered morphological traits and characterization of these plants were performed with an emphasis on plant variants that had semi-dwarf growth habit and still maintained growth vigour.

Materials and Methods

Regeneration of plants and screening for morphological somaclonal variants: Tissue culture was performed as previously reported (Li et al. 2006) in 2005 and 2006 when immature embryos were available in the field. In brief, immature embryos of cv. 'Raleigh' were isolated and cultured on MS medium containing 1 mg/l 2, 4-D and 0.5 mg/l BA, for callus induction and subculture for 4–8 weeks. The regeneration medium was MS medium supplemented with 1 mg/l BA, 0.2 mg/l α-naphthaleneacetic acid (NAA), and 0.5 mg/l gibberellic acids (GA). After 4–6 weeks in regeneration medium, the regenerated plantlets were transplanted to Metro-Mix-200 soil (Scotts Company, Marysville, OH, USA) of 6-inch pots and kept in a lighted culture room. The plantlets were later transferred to a greenhouse with natural daylight and maintained with regular watering and occasional removal of overhanging stolons. Two grams of fertilizer Osmocote (16-4-8) (Scotts Company) were applied to each pot monthly. Plants with altered morphology were further observed for phenotypic

confirmation and later transplanted to the field for additional evaluation.

Selection and characterization of somaclonal variants: Plants were visually screened to identify morphological changes about 3 months after they were grown in the greenhouse. These lines were propagated in trays in the greenhouse, and transplanted in the field in summers of 2006 and 2007. The field trials were conducted at Lake Wheeler Turf Field Lab in Raleigh, NC, USA using a randomized complete block design (RCBD) with three replicates. The soil was Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludults) with pH 6.0. After transplantation, plots were irrigated three times daily for the first month to accelerate establishment and then watered as needed to prevent wilt. The plants were fertilized with 34-0-0 at 49 kg N/ha 4 to 6 weeks after planting, and in summer of the following year. Fungicide 'Heritage' (Syngenta Crop Protection, Greensboro, NC, USA), was sprayed (15.8 g/100 m^2) as needed to prevent grey leaf spot (*Pyricularia grisea*) infection. Weeds were hand removed and no mowing was performed in the first year. Since a majority of the variants grew very slowly or did not grow at all in the field conditions, only lines showing certain growth vigour with development of new stolons and leaves were selected for further evaluations.

The reported somaclonal variants SV15, SV20, and SV27 were transplanted on 22 June 2006. Twenty five to thirty plugs (with stolon length about 15 cm) of each variant were planted within a 0.91 m × 1.22 m plot. Somaclonal variants T4, T5, T7 were planted on Aug 8, 2006. Since it was in late growing season, 0.61 × 0.61 m of sod (produced in soil beds at greenhouse) of each was transplanted into plot centre of 0.91 × 1.22 m plot. Variants 904AT1, 904AT4, 904AT5, 904T1, 904T2, 106T1 and 106T3 were planted on 22 June 2007 using 18 plugs (size similar to the previous group) into 0.61 m^2 plot. Lines 904AT2 and SVC3 were planted on 3 July 2007 with 18 plugs in 1 m^2 plot. In each experiment, 'Raleigh' was planted as a control.

Measurements of the selected somaclonal variant lines were carried out together with the parent control ('Raleigh') when they were well established in the field 3–4 months after transplantation except for SVC3, of which the measurement was conducted in the greenhouse where it was grown in a large soil bed (1.2 × 0.6 m). The following morphological traits were measured from the well-developed stolons in each plot: leaf length, leaf width, and leaf sheath length measured at the third node from the shoot tip (node number refers to counts of only long internodes and not compressed internodes), long internode length and thickness between the third and fourth node, and stolon length from the shoot tip to the fourth node. Inflorescence number per plot was also measured whenever the inflorescences were present.

Statistical analysis: ANOVA was carried out by using SAS software (version 9.1, SAS Institute, Cary, NC, USA). When significant differences (P = 0.05) were observed, the least significant difference test (LSD, Steel et al. 1996) was applied to detect differences among the lines and marked in the tables and figures. Co-efficient variant (CV) is reported as a reference to reflect the range of the variation.

Results

Approximately 7900 plants were regenerated using the improved tissue culture technique. All plants were derived from 'Raleigh', released by North Carolina State University and considered the most cold-tolerant St. Augustinegrass cultivar (Busey et al. 1982). A total of 119 morphological somaclonal variants were visually identified in the greenhouse. However, 100 had little growth months after transplanting to the field, and were not further investigated. Only 19 variants showed reasonable growth and were selected for further evaluations. Among them, 13 had a semi-dwarf growth habit, with shorter leaves and internodes, when compared to 'Raleigh', and grew well. They were SV15, SV20, SV27, T4, T5, T7, 106T1, 904AT4, 106T3, 904T1, 904T2, 904AT1, and 904AT2. Although 106T2 and 904AT3 also had a semi-dwarf growth habit and some growth in the greenhouse, they did not establish well in the field conditions, especially through the winters, and thus no data were collected from these two lines. Two variants (SVC3 and 904AT5) had longer and wider leaves than 'Raleigh'. In addition, a variant, TCo, had variegated leaves (with yellow stripes), and another, TD1, had thicker internodes.

Lines SV15, SV20 and SV27 were approximately 40–50% shorter in stolon and internode length and had 20–25% shorter leaf blades (Fig. 1a and Table 1A) when compared to 'Raleigh'. Lines SV15 and SV27 also had narrower leaf blades than 'Raleigh' (Table 1A). All three lines had significantly more (2–5-fold) inflorescences than 'Raleigh'.

Somaclonal variant lines T4, T5, and T7 also had significantly shorter leaf blades, internodes and stolons (Fig. 1b and Table 1B) compared with 'Raleigh'. The blades of these somaclonal variants were shorter than 'Raleigh' by 20–25%. T7 had the shortest internodes and stolons, which were 2.26 cm and 8.63 cm, respectively, compared to 5.31 and 14.39 cm for 'Raleigh'. In addition, T5 and T7 had significantly thinner stolons compared to 'Raleigh'.

Lines 106T1, 106T3, 904AT1, 904AT2, 904AT4, 904T1, and 904T2 had shorter internode (35–60%) and stolon length (30–60%), respectively, as noted in Table 1C and D. In addition, 904AT2, 904AT4, 904T1-T2, 106T1 and 106T3 showed significantly reduced leaf length, leaf width, and sheath length. For instance, 904AT2 had leaf length, width and leaf sheath shorter than 'Raleigh' by 45%, 27%, and 36%, respectively.

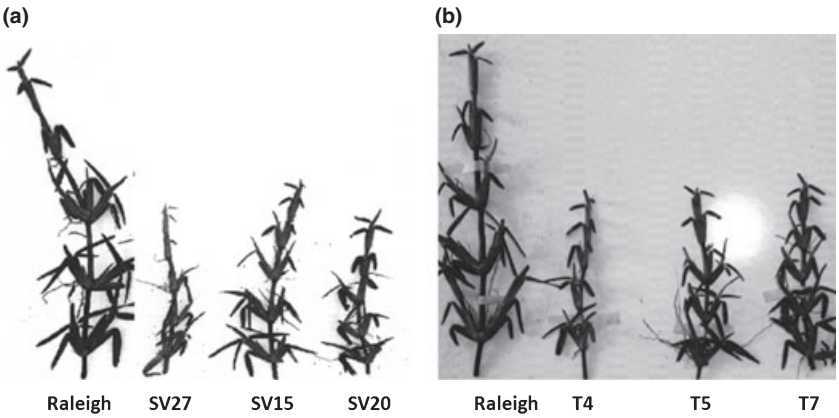

Fig. 1: Representative somaclonal variants with semi-dwarf growth habit. Stolons of six somaclonal variants, together with control 'Raleigh', collected from two field trials (a and b) at NCSU Lake Wheeler Turf Field Laboratory, Raleigh, NC, USA in 2006

Table 1: Morphological characteristics of 15 somaclonal variants in five separate experiments

Lines	LL (cm)	LW (cm)	IL (cm)	ID (mm)	SL (cm)	IN	SSL (cm)
A.							
SV15	1.65^{b}	0.34^{b}	2.59^{b}	2.84^{ab}	10.72^{b}	75.00^{a}	–
SV20	1.74^{b}	0.42^{a}	2.60^{b}	2.69^{b}	11.39^{b}	33.33^{bc}	–
SV27	1.63^{b}	0.34^{b}	2.59^{b}	2.93^{a}	11.21^{b}	32.33^{bc}	–
Raleigh	2.16^{a}	0.46^{a}	4.88^{a}	3.05^{a}	18.53^{a}	16.33^{c}	–
CV%	10.15	15.25	7.63	5.68	6.14	50.30	–
B.							
T4	1.78^{b}	0.45^{a}	2.70^{b}	3.11^{ab}	9.10^{b}	–	–
T5	1.92^{b}	0.45^{a}	2.79^{b}	2.99^{b}	10.13^{b}	–	–
T7	1.84^{b}	0.42^{a}	2.26^{b}	2.98^{b}	8.63^{b}	–	–
Raleigh	2.32^{a}	0.47^{a}	5.31^{a}	3.35^{a}	14.39^{a}	–	–
CV%	6.35	5.17	17.76	5.08	12.50	–	–
C.							
904AT1	1.61^{bc}	0.5^{d}	2.78^{b}	3.03^{dce}	10.94^{b}	–	1.49^{d}
904AT4	1.53^{cd}	0.53^{c}	2.86^{b}	3.29^{ab}	11.21^{b}	–	2.05^{c}
904AT5	2.71^{a}	0.61^{a}	1.76^{d}	3.18^{bc}	8.48^{ed}	–	2.77^{a}
904T1	1.32^{ed}	0.49^{d}	2.49^{c}	2.85^{e}	10.01^{c}	–	2.32^{b}
904T2	1.14^{ef}	0.45^{e}	1.83^{d}	2.97^{de}	7.9^{e}	–	1.57^{d}
106T1	1.73^{bc}	0.54^{c}	2.24^{c}	2.88^{de}	8.81^{d}	–	2.07^{c}
106T3	1.1^{f}	0.49^{d}	2.32^{c}	3.05^{dc}	9.08^{d}	–	1.51^{d}
Raleigh	1.82^{b}	0.57^{b}	4.34^{a}	3.38^{a}	16.23^{a}	–	2.28^{b}
CV%	17.78	6.49	15.10	7.81	9.80		10.00
D.							
904AT2	0.97^{b}	0.44^{b}	1.79^{b}	2.74^{a}	7.61^{b}	–	1.40^{b}
Raleigh	1.75^{a}	0.6^{a}	4.57^{a}	3.3^{a}	16.19^{a}	–	2.19^{a}
CV%	11.21	10.78	14.43	10.46	6.74	–	9.29
E.							
SVC3	4.68^{a}	0.95^{a}	8.17^{a}	3.70^{a}	26.73^{a}	–	4.52^{a}
Raleigh	3.07^{b}	0.83^{b}	7.65^{a}	3.18^{a}	24.23^{a}	–	3.02^{b}
CV%	13.70	4.10	7.16	10.17	3.99	–	6.28

CV, coefficient of variance; LL, leaf blade length; LW, leaf blade width; IL, internode length; ID, internode diameter; SL, stolon length; SSL, sheath length; IN, inflorescence number per plot.
Each value represents means of three replicates. A and B: from field trial of 2006 with 10 individual stolons measured from each replicate. C and D: from field trial of 2007 with 5 individual stolons measured per replicate. E. Each value represents the mean of 6 stolons grown in the greenhouse. Statistical analysis was performed within each group. Values followed by the same letter were not significantly different from each other in the same group according to the LSD (P = 0.05).

Although most of the selected somaclonal variants displayed semi-dwarf growth habit, two variant lines, 904AT5 and SVC3, had significantly longer and wider leaves than 'Raleigh' (Table 1C and E). While SVC3 had internode length similar to 'Raleigh', internodes of 904AT5 were 60% shorter than 'Raleigh'. Interestingly, SVC3 also showed improved freezing tolerance in lab screening (R. Li, unpublished data).

In addition, two other types of morphological changes were observed: line TCo possessed variegated (yellow striped) leaves (Fig. 2a) while line TD1 was found to have significantly thicker internodes than 'Raleigh' (Fig. 2b).

The morphological changes of these variants were stable as demonstrated by their growth performance in the greenhouse, and in the replicated field tests at different years. Some lines have been grown at three locations in North Carolina in field trials and the altered traits have remained stable in these trials (W. C. Reynolds, unpublished data).

Discussion

Breeding through somaclonal variation has yielded many new plant cultivars. However, to our knowledge, this report is the first attempt to use the approach for St. Augustinegrass breeding. The improved tissue culture technique (Li et al. 2006) made it possible to regenerate *in vitro* a large number of plants and thus created a significant pool to identify somaclonal variants. Nineteen somaclonal variants were identified to exhibit altered morphology while still maintaining vigorous growth. Most of the currently used St. Augustinegrass cultivars are considered coarse due to their large leaf blades and long internodes. The somaclonal variant lines with semi-dwarf growth habit we recovered from this project could be employed in breeding efforts for new cultivar development. In addition, line TD1 had thicker stems and line TCo had variegated leaf stripes and may have values for ornamental uses.

One potential problem utilizing somaclonal variation is that some variants are epigenetic effects and result in unstable phenotypes (Kaeppler and Phillip 1993) not suitable for cultivar development. To evaluate the stability of the variant lines, they were grown in replicated field tests and observed for at least 2 years with some being grown at multiple locations with various climate conditions. So far, the altered traits in these lines remained the same during vegetative propagation and when grown in different environments, suggesting that the morphological changes in these variants are stable and could be employed in breeding efforts.

Future research is needed to investigate the cold tolerance and pest resistance of these variants, with emphasis on

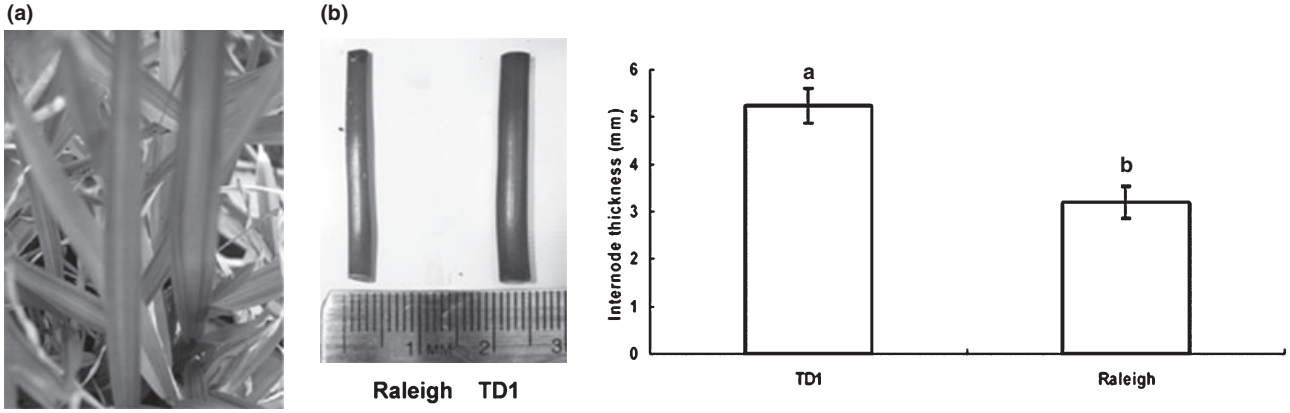

Fig. 2: (a) Somaclonal variant TCo with yellow stripes in its leaves. (b) Somaclonal variant TD1 with thicker stem. Columns and bars represent means and standard errors from 10 stolons of each clone. The difference is significant based on *t*-test (P = 0.05)

resistance to two major pests: St. Augustine decline (SAD, caused by panicum mosaic virus) and southern chinch bug (*Blissus insularis* Barber) (Busey 2003). In addition, since most selected SVs exhibited a semi-dwarf growth habit, they should be compared to commercially available semi-dwarf cultivars such as 'Amerishade' and 'Delmar' (Trenholm et al. http://edis.ifas.ufl.edu/LH010).

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References

Busey, P., 2003: St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze. In: M. D. Casler, and R. R. Duncan (eds), Biology, Breeding, and Genetics of Turfgrasses, 309—330. John Wiley & Sons, Inc, Hoboken, NJ.

Busey, P., T. K. Broschat, and B. J. Center, 1982: Classification of St. Augustinegrass. Crop Sci. **22,** 469—473.

Jain, S. M., 2001: Tissue culture-derived variation in crop improvement. Euphytica **118,** 153—166.

Kaeppler, S. M., and R. L. Phillip, 1993: Tissue culture induced DNA methylation variation in maize. Proc. Natl Acad. Sci. USA **90,** 8773—8776.

Kuo, Y. J., and M. A. L. Smith, 1993: Plant regenerating from St. Augustingrass immature embryo derived callus. Crop Sci. **33,** 1394—1396.

Larkin, P. J., and S. C. Scowcroft, 1981: Somaclonal variation-a novel source of variability from cell culture for plant improvement. Theor. Appl. Genet. **60,** 197—214.

Li, R., A. H. Bruneau, and R. Qu, 2006: In vitro somatic embryogenesis and improved plant regeneration of St. Augustinegrass [Stanotaphrum secundatum (Walt.) Kuntze] by 6-benzyladenine in callus induction medium. Plant Breeding **125,** 52—56.

Steel, R. G. D., J. H. Torrie, and D. A. Dickey, 1996: Principles and Procedures of Statistics, a Biometrical Approach, 3rd edn. McGraw-Hill Companies, New York.

Trenholm, L. E., J. L. Cisar, and J. B. Unruh. St. Augustinegrass for Florida Lawns. http://edis.ifas.ufl.edu/LH010.