Combining Ability for Winter Survival and Turf Quality Traits in St. Augustinegrass

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Abstract. New elite st. augustinegrass cultivars with improved cold tolerance and desirable turf quality are needed for the turf industry, especially in the transitional climatic region of the United States. To efficiently use sources of cold tolerance in a breeding program, an understanding of the genetic control of this trait and its relationship to important turf quality traits is required. Therefore, the objective of this study was to estimate general and specific combining abilities for cold response and turf quality traits. Six diploid genotypes of st. augustinegrass were selected as parents for a diallel mating design without reciprocals and evaluated over 3 years at two locations. The true hybridity of crosses was confirmed using simple sequence repeats (SSR). Combining ability analysis revealed that both general and specific combining abilities were significant across years and locations. Specific combining ability (SCA) was the largest source of genetic variation for winter survival, genetic color, turf density, and end-of-season cover indicating that nonadditive gene effects play a key role in the inheritance of these traits. The parental genotype ‘GF2’ was identified as a promising parent for future breeding efforts as it provided positive general combining ability (GCA) effects for both cold tolerance and turf quality traits, which were not significantly correlated with one another. Lines identified as parental selfs generally showed lower cold response and inferior turf quality than the original parental lines indicating that inbreeding depression can occur in st. augustinegrass. This study provides information regarding the combining ability of cold response and turf quality traits in st. augustinegrass, which will ultimately aid in parental selection for our future breeding efforts.

St. augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntz] is a warm-season, perennial grass species commonly used in the turfgrass industry for its superior shade tolerance and stoloniferous growth habit. However, winter survival is a major limiting factor for the species affecting its area of adaptation and marketability, especially in the transition zone of the United States. In addition, the most cold-tolerant cultivars

currently available often lack aesthetic characteristics, such as finer leaf texture and shorter internode length that are desirable in the market. Therefore, new st. augustinegrass cultivars with improved winter survival and desirable turf quality are needed for the turfgrass industry in the transitional climatic region of the United States.

Studies have reported significant variability in winter survival and freezing tolerance among present-day cultivars (Kimball et al., 2016a, 2016b; Milla-Lewis et al., 2013; Moseley et al., 2010; Reynolds et al., 2009). Cold hardy cultivar, Raleigh (Bateman, 1980), performs consistently well in the upper range of the species’ geographic limits. However, ‘Raleigh’s’ coarser leaf texture and longer internodes when compared with other st. augustinegrass cultivars as well as other warm-season turfgrasses are often undesirable (Reynolds et al., 2009). Somatic variants and gamma-ray mutants of ‘Raleigh’ were developed in 2004 (Li, 2007). In comparison with ‘Raleigh’, they had improved aesthetic

characteristics, but similar to less cold hardiness (Reynolds et al., 2009). On the other hand, available commercial cultivars with good turf quality often have limited cold tolerance. ‘Seville’ (Riordan et al., 1980), a popular cultivar well liked for its semidwarf habit, fine-leaf texture, and dark green color, often exhibits high levels of winterkill (Moseley et al., 2010; Philley et al., 1996; Riordan et al., 1980).

Despite the identification of cold tolerant germplasm, limited progress has been made in breeding for improved winter survival in st. augustinegrass. This is in part due to the complexity of the trait, insufficient genetic knowledge of tolerance components, lack of efficient selection criteria, and limited breeding efforts for this trait. For example, ‘Raleigh’ is still considered an industry standard for cold hardiness in the species 30 years after its release. Previously, Philley et al. (1998) evaluated the inheritance of winter survival in st. augustinegrass with eight diploid genotypes using a diallel analysis. General combining ability was the largest source of variation for winter survival and lethal temperature. The large additive variance indicated that st. augustinegrass should respond to selection (Philley et al., 1998). However, field trials were conducted in Mississippi and may not reflect genotype performance in the upper transition zone. In addition, turf quality was not reported in the study and knowledge of the transferability of turf quality traits from parents to progeny in combination with winter survival in st. augustinegrass is important in the development of new, elite cultivars.

A set of ‘Raleigh’ variants selected for high levels of winter survival as well as improved turf quality (Reynolds et al., 2009) and collections from home lawns in Raleigh, NC, selected for early spring green-up were identified as potential parents for cold tolerant population development. To efficiently use these resources in our breeding program, the objective of this study was to estimate their general and specific combining ability for winter survival and turf quality traits.

Materials and Methods

Six diploid ($2n = 2x = 18$) genotypes of st. augustinegrass were selected as parents for a diallel mating design without reciprocals based on their high levels of winter survival and/or turf quality (Table 1). Reciprocals were not used in this study as Philley et al. (1998) found no maternal effects in their st. augustinegrass heritability study. The six genotypes included: cultivars Raleigh and Seville; experimental lines ‘GF2’, a gamma radiation-derived ‘Raleigh’ mutant, and 106T3, a somaclonal variant of ‘Raleigh’; and accessions ‘C1’ and ‘1800S’, ecotypes collected from old home lawns in Raleigh, NC, selected for early spring green-up (Li, 2007). Crosses were made according to the protocol of Genovesi et al. (2009) during the fall of 2009. One to three SSR markers

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developed by Mulkey et al. (2013) were used to validate that products from crosses were true hybrids following the protocols outlined in that study. Parental selfs identified by SSR markers were included in this study (Table 2).

Three replications of each single cross, parental self, and the six parents were planted in a randomized complete block design in 1-m² plots at the North Carolina State University Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC, and at the Upper Mountain Research Station in Laurel Springs, NC, in June 2011. Plots were mowed weekly at a height of 6.35 cm, fertilized monthly at a rate of 48.8 kg·ha⁻¹ from May to August to accumulate 195.2 kg·ha⁻¹ for a year, and irrigated to avoid drought stress.

Traits were grouped into three categories: cold response including winter survival and spring green-up; turf quality traits, including overall turf quality, leaf texture, genetic color, and turf density; and establishment, including midseason and end-of-season cover. Traits were evaluated over a 3-year period from 2011 to 2014. Spring green-up ratings were taken in April and winter survival ratings were collected in May. Turf quality traits and midseason cover were rated in July, while end-of-season cover was rated in October of each year. Midseason and end-of-season cover of individual plots were rated on a 0% to 100% scale. The remaining traits were evaluated visually on a 1 to 9 scale according to the National Turfgrass Evaluation Program (NTEP, 2012) as follows:

1) spring green-up, 1 = straw brown color and 9 = completely green turf stand; 2) winter survival, 1 = 0% survival and 9 = 100% survival; 3) turf quality, 1 = poor quality and 9 = excellent quality where 5 = minimum acceptable quality; 4) leaf texture, 1 = coarsest texture and 9 = finest texture; 5) genetic color, 1 = light green/yellow and 9 = dark green; and 6) turf density, 1 = lowest density and 9 = maximum density. Note that for each of these rating scales, a higher score indicates superiority.

Genotypic differences were tested using a mixed model (PROC MIXED) in SAS statistical software version 9.4 (SAS Institute, 2011). The models were for parents: $\bar{Y}_{ii} = \mu_p + p_i + \bar{\epsilon}_{ii}$, for selfs: $\bar{Y}_{jj} = \mu_c + 2g_j + s_{jj} + \bar{\epsilon}_{jj}$, and for crosses: $\bar{Y}_{ij} = \mu_c + g_i + g_j + s_{ij} + \bar{\epsilon}_{ij}$ where $\bar{Y}_{ii}$ is the mean for the *i*th parent of the six parents, μ_p is the mean of all parents, p_i is the effect of the *i*th parent, $\bar{Y}_{jj}$ is the mean of the *j*th self of the six parental self, μ_c is the mean of all parental selfs, $2g_j$ is the GCA effect of the *j*th parental self, s_{jj} is the SCA effect of the *j*th parental self, and $\bar{Y}_{ij}$ is the mean for the cross between the *i*th and *j*th parents, μ_c is the mean of all crosses, g_i is the GCA effect of the *i*th parent, s_{ij} is the SCA effect on the cross of the *i*th and *j*th parents ($s_{ji} = s_{ij}$), and $\bar{\epsilon}_{ii}$ and $\bar{\epsilon}_{ij}$ are the mean error terms associated with the respective means. Griffing's (1956) approach, Method 2 (parents, the F₁ generation, and no reciprocals) Model I, was used to analyze general and specific combining abilities for all traits. Mean

separation procedures according to Fisher's protected least significant difference (LSD) values were used to compare parental vs. family means as well as parental vs. self means.

Results and Discussion

The interaction of location by year was significant for winter survival, spring green-up, and genetic color (Table 3). The significant interaction resulted largely from differences in magnitude between years and locations. For example, winter survival was high at Lake Wheeler in 2012 and extremely low at Laurel Springs in 2013 and 2014. However, moderate shifts in cold response as well as genetic color rankings were identified, and both location and year accounted for a large amount of variation in their models. Environmental variation contributed less than genetic effects to the overall variation in the majority of turf quality traits as expected for qualitative characteristics. Similar results were reported in zoysiagrass (*Zoysia* spp.) where large environmental influences were identified for stress-related traits but not for turf quality traits (Schwartz et al., 2009). In addition, large environmental and error effects were also identified for genetic color in Schwartz et al. (2009) who suggested that the use of a handheld normalized difference vegetation index sensor, which has been shown to improve the calculation of color heritability (Kenworthy et al., 2006), could increase realized heritability of genetic color by reducing error variation.

Cold response. Significant variability for cold response was identified among the parental lines in this study (Table 3). According to means separation using Fisher's protected LSD, 'Raleigh's' winter survival was significantly better than all other parental lines (Table 4). The remaining five parental lines could not be significantly distinguished from one another in terms of their winter survival. 'Raleigh' was also the only parent with a significant parental effect for winter survival (Table 5). These findings are consistent with previous studies documenting 'Raleigh's' high level of cold tolerance (Li et al., 2010; Maier et al., 1994; Moseley et al., 2010; Philley et al., 1988). Genotypes 'C1' and '106T3' had the lowest levels of winter survival across locations and years. Although 'C1' is a collection and thus little is known about its genetic makeup, previous studies have reported varying levels of winter survival for '106T3' (Moseley et al., 2010; Reynolds et al., 2009) indicating that this genotype may not be stable across different environments. Somaclonal variants such as '106T3' can be unpredictable in nature and genetically unstable (Dennis et al., 1987; Kaeppler and Phillips, 1993; Lee and Phillips, 1988), and results suggested that further evaluation of the line is needed. Likewise, 'Seville' had higher winter survival than expected for a known cold susceptible cultivar. Previous studies (Kimball et al., 2016a, 2016b; Moseley

Table 1. St. augustinegrass cultivars, ecotypes, and experimental genotypes used in the United States and their breeding information and release date.

No.	Parent	Type	Experimental notation	Source/breeder	Yr released
1	Raleigh	Cultivar	NA	NCSU, Raleigh, NC	1983 ²
2	1800S	Ecotype from NCSU collection	1800S	NCSU, Raleigh, NC	NA ³
3	GF2	'Raleigh' Mutant	GF2	NCSU, Raleigh, NC	NA ³
4	Seville	Cultivar	S-6-68-516	Pursely, Inc.; T.P. Riordan; O.M. Scotts & Sons	1978 ³
5	C1	Ecotype from NCSU collection	C1	NCSU, Raleigh, NC	NA ³
6	106T3	'Raleigh' somaclonal variant	106T3	NCSU, Raleigh, NC	NA ³

NCSU = North Carolina State University.

²Bateman, 1980.

³NA = Not applicable. Experimental germplasm under development.

³Riordan et al., 1980.

Table 2. Percentage of selfed progeny per cross and simple sequence repeat (SSR) markers used to verify hybridity.

Cross combinations ²	Selfed progeny (%)	SSR markers
106T3 × 1800S	63	SAM 1709, SAM 23965, SAM 5295
106T3 × C1	63	SAM 5295
106T3 × GF2	50	SAM 21225
106T3 × Seville	63	SAM 5295, SAM 7483, SAM 12183
C1 × 1800S	50	SAM 17792, SAM 21225
C1 × GF2	50	SAM 21225
C1 × Seville	63	SAM 5258, 12183, SAM21225
GF2 × 1800S	50	SAM 17792, SAM 7637, SAM 21225
Raleigh × 106T3	63	SAM 5295
Raleigh × 1800S	50	SAM 17792, SAM 7637, SAM 21225
Raleigh × C1	50	SAM 21225
Raleigh × GF2	50	SAM 5295, SAM 21225
Raleigh × Seville	63	SAM 2460, SAM 1512, SAM 11067
Seville × 1800S	50	SAM 434, SAM 1709, SAM 21225
Seville × GF2	63	SAM 21225, SAM 7637, SAM 4343

²A total of eight crosses were produced per parental combination.

Table 3. Analysis of variance for the main effects (location, year, parents, selfing, and crosses) and the interactions of a nonreciprocal mating design for winter survival, spring green-up, turf quality, genetic color, leaf texture, turf density, midseason cover, and end-of-season cover from data collected at the Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC, and the Upper Mountain Research Station in Laurel Springs, NC, from 2011 to 2014. Estimations of general combining ability (GCA) and specific combining ability (SCA) were determined among crosses for each parental genotype.

Source	df	Winter survival	Spring green-up	Turf quality	Genetic color	Leaf texture	Turf density	Midseason cover	End-of-season cover
Location	1	577.19***	714.50***	16.43**	13.39	6.79**	66.53***	11,058.01***	32,792.54***
Year	2	746.73***	325.45***	56.92***	21.85**	6.43**	39.73***	60.33	57.54
Replication	2	13.08***	7.27	3.43	1.06	1.01	23.50***	1,980.84*	8,612.05***
Parents	6	45.62***	69.49***	32.67***	23.72***	2.45*	54.45***	5,534.68***	6,904.26***
Selfs	5	3.40	5.30	20.75***	13.69***	10.68***	31.62***	6,613.43***	5,339.67
Crosses	14	23.49***	17.29***	14.87***	9.24***	5.31***	20.36***	2,393.88***	4,892.72***
GCA	5	19.36***	43.09***	27.76***	1.65	22.56***	29.81***	4,243.47***	5,214.35***
SCA	9	30.47***	39.75***	23.48***	6.57***	18.24***	31.83***	1,905.71***	5,425.18***
Parents vs. crosses	1	0.09	0.07	4.21	0.3	0.01	5.21	1,374.97	2,632.53
Parents vs. selfs	1	107.64***	257.86***	79.84***	0.08	139.77***	116.48***	7,967.81***	12,549.01**
Location × year	2	141.34***	157.94***	0.25	29.32***	0.91	4.08	200.23	212.68
Location × replication	2	1.66	0.41	0.64	13.82**	0.38	9.83*	62.80	2,688.82
Location × parents	6	22.21***	37.18***	11.82***	11.75***	0.77	19.82***	5,413.33***	5,711.20***
Location × crosses	14	6.03***	5.19	5.58**	2.41	3.06***	7.31**	681.03	3,745.14***
Year × replication	4	1.48	3.86	0.85	1.71	1.06	0.34	203.46	212.16
Year × parents	12	17.63***	18.58***	2.70	2.25	6.97***	1.76	2,210.54*	2,323.79*
Year × crosses	28	6.51***	5.13	2.75	2.35	0.82	3.2	1,149.62	1,325.51
Location × year × replication	4	4.69*	2.48	0.44	1.6	1.48	15.81**	2,348.05	2,437.01
Location × year × parents	12	8.30***	20.46***	6.47**	1.7	1.73	2.16	1,578.49	1,696.39
Location × year × crosses	28	4.67***	2.53	2.11	2.63	0.58	1.52	795.35	847.25

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

Table 4. Performance of six st. augustinegrass parents and their cross combinations for winter survival, spring green-up, turf quality, genetic color, leaf texture, and turf density (rated on a 1–9 scale), and midseason cover and end-of-season cover (rated on a 0% to 100% scale) from data collected at the Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC, and the Upper Mountain Research Station in Laurel Springs, NC, from 2011 to 2014.

ID	No. of lines	Winter survival	Spring green-up	Turf quality	Genetic color	Leaf texture	Turf density	Midseason cover	End-of-season cover
Raleigh	4	4.86 a ^z	2.96 ab	6.39 bcd	6.61 bc	5.9 efg	6.27 bcd	78.96 cdef	93.96 ab
1800S	4	3.56 cde	2.79 ab	6.64 b	6.87 b	6.58 ab	6.62 bc	64.58 ghi	85.42 abcd
GF2	4	3.46 cde	3.75 a	6.26 bcde	6.45 bcd	6.11 cdef	5.87 cdef	54.79 ij	80.94 bcde
Seville	4	3.46 cde	1.64 cde	7.56 a	7.43 a	6.27 abcdef	7.58 a	98.96 a	87.29 abcd
C1	4	3.24 defg	2.31 bcd	5.48 fghi	5.97 defg	5.89 efg	5.05 fghi	57.93 hij	71.35 efgh
106T3	4	3.08 efgh	2.18 bcd	5.06 ghi	6.01 def	6.51 abc	4.62 ghi	44.79 j	64.17 gh
106T3 × 1800S	3	3.61 cd	2.29 bcd	5.65 efg	5.96 defg	5.87 fg	5.28 efg	89.44 abc	80 cde
106T3 × C1	3	2.66 hij	1.5 cde	4.88 hi	5.64 fg	5.33 h	4.48 hi	73.33 defgh	63.47 gh
106T3 × GF2	4	3.57 cde	2.32 bc	5.93 def	6.4 bcd	6.55 ab	5.59 def	79.49 cdef	76.88 def
106T3 × Seville	3	3.8 bc	2.29 bcd	6.54 bc	6.65 bc	6.17 bcdef	6.83 b	90.28 abc	95.69 a
C1 × 1800S	4	4.22 b	2.68 b	6.68 b	6.73 bc	6.57 ab	6.55 bc	79.79 cde	90 abc
C1 × GF2	4	2.28 j	1.35 ed	—	4.85 h	5.67 gh	4.29 i	76.46 defg	61.67 h
C1 × Seville	3	2.65 hij	1.04 e	5.69 efg	5.39 g	6.06 defg	5.21 efgh	95.83 ab	75.83 defg
GF2 × 1800S	4	2.81 ghi	1.76 cde	5.35 gh	6.08 def	5.98 efg	5.12 fgh	66.75 fghi	66.17 fgh
Raleigh × 106T3	3	3.31 cdef	1.93 cde	5.97 cdef	5.88 efg	6.4 abcd	5.75 def	83.06 bcd	86.81 abcd
Raleigh × 1800S	4	2.92 fghi	1.63 cde	5.13 gh	5.76 fg	5.44 h	4.73 ghi	69.58 efgh	75.42 defg
Raleigh × C1	4	3.08 efgh	2.13 bcd	5.02 hi	5.93 defg	6.1 def	4.85 ghi	72.71 defgh	68.75 efgh
Raleigh × GF2	4	2.74 hi	1.54 cde	5.32 gh	5.79 efg	5.99 efg	5.24 efgh	56.41 ij	66.46 fgh
Raleigh × Seville	3	2.87 gfghi	1.43 cde	6.49 bcd	6.29 cde	6.6 a	5.89 cde	90.83 abc	77.5 def
Seville × 1800S	4	2.49 ij	1.49 cde	5.64 fghi	6.02 def	6.31 abcde	5.18 efgh	75.42 defg	69.46 efgh
Seville × GF2	3	2.46 ij	1.02 e	6.59 b	6.82 bc	6.66 a	6.03 cd	80.28 cde	68.61 efgh

^zMeans with the same letter are not significantly different from one another.

et al., 2010; Philley et al., 1995) have reported varying winterkill and freeze damage in ‘Seville’ and suggest that ‘Seville’ is another genotype whose performance is not consistent across environments.

For spring green-up, ‘GF2’, ‘Raleigh’, and ‘1800S’ were the top performers. ‘Seville’ was the slowest to green-up. As the best and worst performers, ‘GF2’ and ‘Seville’ had significant parental effects for spring green-up, respectively (Table 5). A significant, positive correlation (0.63) between winter survival and spring green-up was detected between parental lines as well as between crosses in this study (Table 6) indicating that a genotype able to withstand cold temperatures in winter will green up

faster in the spring than a genotype severely affected by winterkill. Little has been reported on st. augustinegrass rate of spring green-up in the literature with the exception of limited data from the NTEP (2012).

Across all locations and years, the GCA and SCA effects were both significant sources of variation for winter survival and spring green-up (Table 3). Significant GCA effects indicated parents of genotypes contributed differently to responses in hybrid combinations as well as the involvement of additive gene effects in response to winter survival and spring green-up. However, for winter survival, SCA effects accounted for three times ($\approx 60\%$) more of the total genotypic variance than GCA effects ($\approx 20\%$). The high

SCA effects for winter survival suggested that nonadditive gene effects (i.e., dominance and epistasis) play an important role in cold response in the species. For example, while ‘Raleigh’ had the largest parental effect for winter survival (0.82), it had a significant negative GCA effect (-0.72) and a non-significant, mainly neutral self-effect (-0.05) (Table 5). Because ‘Raleigh’ was no longer distinguishable from other parents on selfing, these results suggest that while dominance could contribute to winter survival, strong heterotic effects and epistatic interactions could also play a key role in the genetic control of this trait. Conversely, Philley et al. (1998) found that the largest portion of variance was additive for lethal temperature

Table 5. Estimates of parental, self, and general combining ability (GCA) effects for winter survival, spring green-up, turf quality, genetic color, leaf texture, turf density, midseason cover, and end-of-season cover from data collected at the Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC, and the Upper Mountain Research Station in Laurel Springs, NC, from 2011 to 2014.

Line	Winter survival			Spring green-up			Midseason cover			End-of-season cover		
	P	S	GCA	P	S	GCA	P	S	GCA	P	S	GCA
Raleigh	0.82***	-0.05	-0.72***	0.08	-0.02	-1.26***	0.66	7.94**	8.29*	5.76	-2.39	-6.72
106T3	-0.19	-0.09	0.05	-0.34	-0.05	-0.23	-19.62***	-9.73**	10.01***	-10.35*	-8.57*	2.10
C1	-0.33	0.24**	-0.51***	-0.30	0.15	-0.70	-1.06	3.57	9.20**	-5.35	6.33*	-8.96*
Seville	-0.14	0.01	0.96***	-1.25***	-0.20	1.45***	26.77***	17.48***	-2.96	5.35	10.33***	21.79***
GF2	-0.09	-0.06	0.66**	1.56***	-0.22	1.71***	-8.23	3.22	-35.27***	0.63	1.63	-3.40
1800S	-0.06	-0.06	-0.44**	0.25	0.32	-0.97***	1.49	-22.48***	10.73**	3.96	-7.34	-4.81

Line	Turf quality			Genetic color			Leaf texture			Turf density		
	P	S	GCA	P	S	GCA	P	S	GCA	P	S	GCA
Raleigh	-0.23	-0.36*	-0.95***	-0.06	-0.36*	-0.97***	-0.38*	-0.33**	-0.11	-0.20	-0.38*	-0.98***
106T3	-0.69	-0.23	-0.24*	-0.48*	0.05	-0.31***	0.35*	0.12	-0.06	-0.96**	-0.51*	-0.17
C1	-0.53	-0.01	-0.74***	-0.54*	-0.17*	-0.77***	-0.35*	-0.16*	-0.16	-0.64*	-0.11	-0.78***
Seville	1.20***	0.91***	2.07***	1.02***	0.56***	1.67***	0.09	0.68***	0.48**	1.44***	1.04***	2.10***
GF2	-0.09	0.02	0.63*	-0.18	-0.12	1.14***	-0.03	0.06	0.06	-0.14	-0.01	0.67*
1800S	0.35	-0.33	-0.77***	0.24	0.04	-0.76***	0.33*	-0.36*	-0.22	0.50	-0.03	-0.83**

P = parental effect; S = self-effect.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

Table 6. Pearson correlation coefficients between parental lines and between crosses for winter survival, spring green-up, turf quality, leaf texture, genetic color, turf density, midseason cover, and end-of-season cover from data collected at the Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC, and the Upper Mountain Research Station in Laurel Springs, NC, from 2011 to 2014.

	Winter survival	Spring green-up	Turf quality	Leaf texture	Genetic color	Turf density	Midseason cover
Parental lines							
Spring green-up	0.49**						
Turf quality	-0.49**	0.26					
Leaf texture	-0.28	-0.17	0.54**				
Genetic color	-0.02	0.00	0.50**	0.14			
Turf density	-0.26	0.21	0.83***	0.56**	0.29		
Midseason cover	-0.46	0.55*	0.80***	0.25	0.67**	0.73**	
End-of-season cover	-0.30	0.07	0.52**	0.09	0.54***	0.47**	0.83***
Crosses							
Spring green-up	0.79***						
Turf quality	0.11*	0.22***					
Leaf texture	-0.10*	-0.06	0.30***				
Genetic color	0.06	0.06	0.48***	0.13			
Turf density	0.44***	0.44***	0.79***	0.19***	0.38***		
Midseason cover	0.55***	0.46***	0.44***	0.08	-0.03	0.58***	
End-of-season cover	0.72***	0.56***	0.37***	-0.02	0.22***	0.67***	0.55***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

and winter survival, suggesting that st. augustinegrass should respond well to selection. The differences in results of the two studies may reflect the large environmental differences between testing regions as well as the germplasm used. In addition, this study has demonstrated (see below under selfing rates section) the importance of confirming true hybridity in crosses of st. augustinegrass as selfing is an important issue to consider in these types of studies.

There was a large range of significant positive and negative SCA effects for winter survival and spring green-up for the majority of crosses (Table 7). In general, SCA estimates indicated that crosses with good winter survival also had faster spring green-up, which is consistent with the high correlations identified between family means for these traits (Table 6). The cross 'Seville' × 'GF2' had larger SCA effects for winter survival and spring green-up than any other cross (Table 7). The effect of this particular cross largely contributed to the significantly high SCA effects identified for winter survival and spring green-up and

provide evidence that a more complex type of inheritance than additive gene effects may be involved.

Turf quality. Significant variability for turf quality traits was identified among the parental lines in this study (Table 3). Although '1800S' had the finest leaf texture, 'Seville' outperformed all parental lines in turf quality, turf density, and genetic color (Table 4). Turf quality provided excellent separation of the genotypes (Table 3) with the most winter hardy genotypes forming two separate groups based on Fisher's LSD values. The genotypes 'Raleigh', '1800S', and 'GF2' appeared to have an intermediate turf quality between 'Seville' and the lowest parental performers, 'C1' and '106T3'. Similar results can be seen for genetic color and turf density, while, as expected, leaf texture had the lowest amount of variability between the parental lines (Table 4).

Across all locations and years, GCA and SCA effects were significant sources of variation for turf quality traits (Table 3). Additive gene effects appear to play a larger role than nonadditive effects for turf quality

and leaf texture, while SCA or nonadditive effects contributed twice as much to the overall variation than GCA effects for genetic color (Table 3). The magnitude of SCA effects was slightly higher than GCA effects for turf density as well. These results indicated that while turf quality traits can be improved through selecting parents with significant GCA values, progeny testing of specific parental combinations could provide more value to our breeding program. For example, the cross 'Seville' × 'GF2' had the largest SCA values for turf quality, genetic color, and turf density along with high SCA values for winter survival and spring green-up (Table 7), and has proven to be an excellent st. augustinegrass cross producing progenies with superior performance.

The cultivar Seville had the highest positive GCA effect for all turf quality traits (Table 5). The majority of winter hardy genotypes provided a negative or neutral GCA effect, with the exception of 'GF2' on overall turf quality, genetic color, and turf density. 'GF2' also had a positive GCA effect for both winter survival and spring green-up. Previous studies have reported better establishment rates, qualitative characteristics, and similar winter survival of 'GF2' when compared with 'Raleigh' (Li, 2007; Reynolds et al., 2009). This genotype showed the most promise as a parental line to transfer both winterhardiness and turf quality traits into new breeding lines, especially with the low correlations identified between these two types of traits (Table 6).

Establishment. Significant variability for midseason and end-of-season cover ratings was identified among the parental lines in this study (Table 3). For midseason cover, 'Seville' had significantly better cover than other parental lines at 99%, which dropped to ≈87% at the end of the season (Table 4). This may indicate that low temperature injury was already beginning to occur in 'Seville' during the fall, especially as it was the only genotype whose cover ratings dropped between rating times. 'Raleigh' had the second

Table 7. Estimates of the specific combining ability of 15 crosses in st. augustinegrass for winter survival, spring green-up, turf quality, leaf texture, genetic color, turf density, midseason cover, and end-of-season cover traits evaluated at the Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC, and the Upper Mountain Research Station in Laurel Springs, NC, from 2011 to 2014.

Cross	Winter survival	Spring green-up	Turf quality	Leaf texture	Genetic color	Turf density	Midseason cover	End-of-season cover
106T3 × 1800S	0.43*	0.75**	0.27	-0.04	0.29	0.18	-0.19	2.00
106T3 × C1	-0.99***	-1.03**	-1.23***	-0.75***	-0.90***	-1.51***	-7.00	-19.49***
106T3 × GF2	-0.04	-0.19	-0.21	0.4**	-0.06	-0.36	-0.06	-6.12
106T3 × Seville	0.18	-0.21	0.40	0.02	0.24	0.90**	11.38*	12.89**
C1 × 1800S	0.43*	0.75**	0.27	-0.04	0.29	0.18	-0.19	2.00
C1 × GF2	-1.00***	-0.91**	-0.99***	-0.25	-1.00***	-1.01**	6.63	-12.99**
C1 × Seville	-0.41*	-1.00**	0.05	0.01	-0.57**	-0.08	17.74**	4.09
GF2 × 1800S	-1.65***	-2.63***	-1.33**	-1.13***	-1.62***	-1.57**	21.67*	-14.14
Raleigh × 106T3	0.42*	0.68**	0.78***	0.37*	0.43*	0.80**	-4.14	10.72*
Raleigh × 1800S	0.51**	1.11**	0.51*	-0.40*	0.76**	0.46	-17.06**	6.65
Raleigh × C1	0.78***	1.34***	0.35	0.21	0.94***	0.54*	-11.01*	4.63
Raleigh × GF2	-0.77**	-1.63***	-0.59*	-0.17	-0.99***	-0.47	15.6**	-3.72
Raleigh × Seville	-0.94***	-1.5***	-1.05***	-0.01	-1.15***	-1.33***	16.61**	-18.28**
Seville × 1800S	-1.60***	-1.74***	-1.9***	-0.19	-1.57***	-2.01***	0.03	-26.98***
Seville × GF2	2.77***	4.46***	2.50***	0.18	3.05***	2.53***	-45.76***	28.28**

* $P > 0.05$, ** $P > 0.01$, *** $P > 0.0001$.

highest cover rating during midseason and the best cover rating at the end of the season (Table 4). Interestingly, significant positive correlations were found between winter survival and both midseason (0.55) and end-of-season (0.72) cover ratings when comparing crosses but nonsignificant, negative correlations between these traits were identified when comparing parental lines (Table 6). It appears that these negative correlations between parental traits were most likely attributed to 'Seville's' high cover ratings and intermediate winter survival (Table 4). The positive correlations between winter survival and cover ratings when comparing crosses indicated that a full turf stand, which is indicative of good overall plant health, appeared to aid a genotype's ability to withstand cold temperatures. In addition, full midseason cover indicates fast establishment rates leading to reduced soil exposure for weed competition in newly established turf stands (Busey and Myers, 1979), which is important for new cultivars in the turfgrass industry.

Across all locations and years, GCA and SCA effects were both significant sources of variation for both cover ratings (Table 3). Large positive and negative differences in GCA effects were identified for parental lines. 'Seville' had the largest GCA effect for end-of-season cover followed by '106T3'. '1800S', '106T3', 'C1', and 'Raleigh' which had significantly large GCA effects on midseason cover but not for end-of-season cover (Table 5).

Overall cross performance. As mentioned above, the cross 'Seville' × 'GF2' proved to be an excellent cross with high performance (Table 4) and significantly high SCA effects (Table 7) for a majority of traits. The crosses '106T3' × '1800S', '106T3' × 'GF2', and '106T3' × 'Seville' also had good winter survival, fast spring green-up, good turf quality, and fast establishment rates (Table 4). In addition, the cross 'C1' × '1800S' had very good spring green-up and turf quality. Cross combinations with poor performance were identified as well. The cross 'Raleigh' × 'GF2' produced low performing progenies across

Fig. 1. Least significant means and mean separation between parental and selfed lines for a = winter survival; b = turf quality, spring green-up, genetic color, leaf texture, turf density, midseason cover, and end-of-season cover.

the winter survival and turf quality traits. As 'GF2' is a gamma-ray derived mutant of 'Raleigh', it is possible that these two parents are highly similar to one another genetically resulting in high levels of inbreeding

depression within crosses between the two.

Selfing rates and inbreeding depression. Although st. augustinegrass is widely known as a predominately outcrossing species, this

research identified high rates of selfing using SSR markers to confirm parentage. This reflects the importance of using molecular markers to validate the true hybridity of crosses. Studies in common bermudagrass [*C. dactylon* (L.) Pers. var. *dactylon* (Tan et al., 2015)] and zoysiagrass (*Zoysia* spp.) (Guo et al., 2014) have identified high outcrossing rates in these species with the use of molecular markers. However, crosses in these studies were generated in open-pollinated fields where pollen was not limited. The high selfing rates observed in controlled crosses in the current study (Table 2) may be the result of a deficiency in crossing protocols, but could also indicate an underestimation in the levels of self-pollination occurring in the species, especially when pollination is limited to a turf stand of the same genotype. It is important to ascertain the cause of these high selfing rates of controlled crosses as they can negatively affect the progress and efficiency of a breeding program.

Previous studies have reported the negative impacts of inbreeding in st. augustinegrass (Atilano and Busey 1983; Busey, 2003). Comparison of the parental and self means and effects indicate that, in general, inbreeding depression impacted most genotypes negatively across traits (Fig. 1). However, depending on the trait, the selfed progeny of some parents showed little to no change, and a few actually showed improvement. For winter survival, all parental means were significantly higher than their selfed counterparts with the exception of 'C1', indicating that selfing had a negative impact on winter survival of progeny for most parents (Fig. 1A). Similar results were seen for spring green-up, turf quality traits, and establishment (Fig. 1). Little change was seen in the leaf texture of parental lines and their selfs.

Interestingly, 'C1' and '106T3' progeny after selfing improved for all traits except for leaf texture in '106T3' selfs (Table 7). Progeny from neither parental genotype was severely affected by selfing and selfed progeny performed better for several traits (Fig. 1). Although 'C1' is an ecotype and little is known about its historical pedigree, as a somaclonal variant of 'Raleigh', '106T3's' improvement upon selfing provides further evidence that the genotype is genetically unstable. This also indicates that selfing may help stabilize somaclonal variants in st. augustinegrass and improve their performance in the field for important turf quality traits.

Conclusions

Significant variation was observed between parental lines and the progeny means of crosses for cold response and turf quality traits. The GCA and SCA effects were both significant for all traits, whereas SCA effects were greater for winter survival, genetic color, turf density, and end-of-season cover indicating that nonadditive gene effects may be more important than additive gene effects for these traits. Although 'Raleigh' exhibited a negative GCA effect for winter survival, 'GF2' had a positive GCA effect for both this trait and turf quality and shows promise as a parental line for future breeding efforts in North Carolina.

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