The Effect of *PcGA2ox* Overexpression on Creeping Bentgrass (*Agrostis stolonifera* L.): Performance under Various Light Environments

Aneta K. Studzinska¹, David S. Gardner, and James D. Metzger

Department of Horticulture and Crop Science, 2021 Coffey Road, The Ohio State University, Columbus, OH 43210-1086

David Shetlar

Department of Entomology, 2501 Carmack Road, Columbus, OH 43210

Robert Harriman

The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041

T. Karl Danneberger

Department of Horticulture and Crop Science, 2021 Coffey Road, The Ohio State University, Columbus, OH 43210-1086

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Abstract. Turf grown in shade exhibits increased stem elongation. Dwarfism could improve turfgrass quality by reducing elongation. The purpose of this study was to examine the effect of *GA2-oxidase* (*GA2ox*) overexpression on creeping bentgrass (*Agrostis stolonifera* L.) performance under restricted light conditions and low mowing heights. Greenhouse studies were conducted at The Ohio State University, Columbus, OH, from 1 Sept. to 31 Oct. in both 2008 and 2009. Two experimental lines, Ax6548 and Ax6549, transformed with *CP4 EPSPS* and *PcGA2ox* gene; and a nontransformed control (NTC) was subjected to four light environments: full sun, reduced red to far red light ratio (R:FR), neutral shade [reduced photosynthetic photon flux (PPF)], and canopy shade (reduced PPF and R:FR). Turf was evaluated every 10 days for color and percent coverage. *GA2ox* overexpression resulted in darker green color in both transgenic lines under all light treatments as compared with NTC plants. No differences in overall turfgrass coverage were noted in full sun conditions among the lines. A significant decrease in turf coverage occurred for all shade treatments regardless of line. However, Ax6549 decreased the least. Overall data indicated that *GA2ox* overexpression can improve quality of turfgrass under reduced light conditions.

Creeping bentgrass (*Agrostis stolonifera* L.) is a turfgrass species highly suitable for use on golf course tees, greens, and fairways. As a result of its ability to provide exceptional quality playing surfaces when mowed short, it is used worldwide. Because of golf course construction features, bentgrass is often maintained under reduced light conditions. Although it tolerates partial shading, it grows best in full sunlight (Beard, 1973; Bell and Danneberger, 1999).

Turfgrasses can be subjected to both natural and artificial (neutral) shade from vegetation and building structures, respectively.

While under neutral shade, turfgrasses respond to reduced light intensity; shade under vegetation canopy can reduce light intensity and alter spectral composition, which act in concert to determine turf performance. Reduction of PPF was shown to induce excessive vertical shoot growth in turfgrass plants at the expense of tiller formation and lateral spread, thereby resulting in a poor density of the turfgrass stand (Bell and Danneberger, 1999; Dudeck and Peacock, 1992; Koh et al., 2003; Wherley et al., 2005). Moreover, turfgrasses grown in low PPF environments were characterized by longer, thinner, and more succulent leaves (Allard et al., 1991; Wherley et al., 2005; Wilkinson and Beard, 1974). Alteration in spectral composition and specifically reduced R:FR further contribute to aforementioned morphological changes (Casal et al., 1990; Dudeck and Peacock, 1992; Frank and Hofman, 1994; Wherley et al., 2005). However, Wherley et al. (2005) reported that leaves of plants grown under low PPF but high R:FR were wider compared with those grown under low PPF and low R:FR environments.

Under golf course conditions, shaded creeping bentgrass is maintained by frequent mowing at reduced heights. As a result, the bentgrass suffers decreased photosynthetic capacity, which ultimately leads to poor stand quality. Wilson (1997) suggested that when selecting species for shaded environments, the focus should include: compact growth morphology, relatively insensitive to changes in PPF and R:FR, and lax, horizontally oriented leaves.

Plant responses to light stimuli, including light quality, quantity, and duration, are in part mediated by gibberellins (GAs) (Hedden and Kamiya, 1997; Sponsel and Hedden, 2004). GAs are phytohormones that are involved in many developmental processes including stem elongation (Davies, 2007). GAs act by inducing genes involved in cell elongation and division (Sun, 2004). GA levels can be reduced in plants through application of growth regulators or biotechnological manipulation of genes involved in the biosynthetic pathway (Busov et al., 2003; Coles et al., 1999; Tan and Qian, 2003).

Among GA-inhibiting growth regulators, trinexapac-ethyl (TE) suppresses vertical growth and improves overall turf quality under low light conditions (Goss et al., 2002; Steinke and Stier, 2003). TE competitively inhibits the conversion of GA20 to GA1 × 3-β-hydroxylase, reducing leaf cell elongation (Adams et al., 1992) but not cell division (Ervin and Koski, 2001). However, to ensure consistent and lasting effects, frequent applications of TE are required.

In plants, inactivation of bioactive gibberellins GA1 and GA4 is ensured by GA2-oxidases (GA2ox) that catalyze their 2β-hydroxylation yielding GA8 and GA34 (Hedden and Proebsting, 1999). Overexpression of *OsGA2ox1* in rice caused a dwarf phenotype with leaves that were darker green, shorter, and wider than those of the wild-type plants and adversely affected development of reproductive organs (Sakamoto et al., 2001). A similar phenotype was obtained by expressing *GA2ox* in transgenic tobacco plants (*Nicotiana tabacum*) (Biemelt et al., 2004; Schomburg et al., 2003), poplar trees (*Populus tremula* × *Populus alba*) (Busov et al., 2003), and Arabidopsis (*Arabidopsis thaliana*) (Radi et al., 2006). Overexpression of *AtGA2ox1* in bahiagrass (*Paspalum notatum* L.) produced a semidwarf phenotype with increased tillering, delayed flowering, and shorter inflorescence, thus enhancing its overall quality (Agharkar et al., 2007).

Creeping bentgrass plants containing the runner bean (*Phaseolus coccineus*) *GA2-oxidase* gene (*PcGA2ox*) have been developed, and through preliminary greenhouse and field studies (Yan, 2005), superior lines were chosen. These superior lines were characterized by more horizontal growth habit, inhibited vertical growth, internode extension, and leaf growth when grown under restricted light conditions. The objective of this study was to determine the effect of genetically induced dwarfism on creeping bentgrass performance under different shade

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¹To whom reprint requests should be addressed; e-mail studzinska.1@buckeyemail.osu.edu.

treatments while being maintained at a low mowing height.

Materials and Methods

Plant material and growth conditions. Transgenic creeping bentgrass (*Agrostis stolonifera* L.) plants were previously developed from the callus of cultivar Crenshaw by Scotts Miracle-Gro Company (Marysville, OH). Based on initial studies conducted by Yan (2005), two superior lines were selected for further research, Ax6548 and Ax6549, transformed with runner bean (*Phaseolus coccineus*) GA2-oxidase gene (*PcGA2ox*), and *Agrobacterium* CP4-EPSPS gene as a selective marker. NTC plants were included in this study as a control. All transgenic and nontransgenic plants were propagated vegetatively in 12-cm diameter pots using Metro-mix 350™ (Scotts Miracle-Gro Company) as a growing medium. Plants were propagated in June 2008 and 2009 and left for 3 months to establish full pot cover. During the establishment and duration of the study, plants were maintained at 15 mm height. In 2008, plants were trimmed every 10 d and in 2009 every 5 d.

Plants were fertilized at the rate of 0.2 kg nitrogen per 92.9 m² using 100 ppm solution

of 20N–10P₂O₅–20K₂O fertilizer (Scotts/Sierra, USA). Applications of chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) and iprodione [3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide] fungicides and spinosad (including Spinosyn A and Spinosyn D), imidacloprid [1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine], cyfluthrin [cyano(4-fluoro-3-phenoxyphenyl)-methyl 3-(2,2-dichloroethenyl)], and bifenthrin (2-methyl-3-phenylphenyl)methyl (1S,3S)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl-2,2-dimethylcyclopropane-1-carboxylate] insecticides were done monthly to prevent pest occurrence. Plants were irrigated to prevent wilting.

Treatments and experimental design. Research was conducted at The Ohio State University, Columbus, OH. Two greenhouse experiments were performed from 1 Sept. to 31 Oct. 2008 and 2009. Both years, plants were randomly assigned to four irradiance treatments: full sun (control), photosensitive blue polyethylene film (reduced R:FR), black shade cloth (reduced PPF), and both blue polyethylene film with black shade cloth (reduced PPF and R:FR) (Table 1). The experimental design was a split plot with three replicates with irradiance treatments as main plots and turfgrass genotypes as subplots.

PPF measurements were taken every 15 min using cosine corrected photosynthetically active radiation light sensors (Spectrum Technologies, Plainfield, IL). Data loggers (WatchDog 225™; Spectrum Technologies) were used to monitor air temperature and relative humidity. Additionally, spectral distribution was measured in the range of 400 nm to 800 nm, and R:FR was calculated from the wavelengths of 650–670 and 720–740 nm (Brutnell, 2006). Measurements were taken using a LI-1800 spectroradiometer (LI-COR Bioscience, Lincoln, NE).

Data collection and statistical analysis. Turf was examined every 10 d, starting from Day 1, for color and coverage. Color was rated visually using a 1–9 scale with 1 representing brown, 5 yellow, and 9 blue–green. Pot coverage was rated visually as a percentage of maximum coverage. Only living tissue was considered during the assessment. To test the effect of light, gene transformation, and their interaction, data from both experiments were analyzed by analysis of variance for split plot design using PROC MIXED (SAS Institute, Cary, NC). SEMs were used to calculate least significant differences at *P* = 0.05 for means separation.

Results and Discussion

As a result of the differences in cutting frequency, data from the two studies were analyzed separately. In both years, light had a significant effect on turfgrass color and density. A light-by-line interaction was observed. Line by itself did not have a significant effect on density in 2008. A significant (*P* = 0.05) interaction between line and sampling time occurred both years and in regard to both density and color ratings.

Table 1. Light conditions for Sept. to Oct. 2008 and 2009.

Light treatment	PPF ^a [mol photons/m ² /d] (% of full sun value)		R:FR ^b 2008/2009
	2008	2009	
Full sun	16.6 (100%)	16.5 (100%)	1.28
Reduced R:FR	11.8 (70%)	10.3 (62%)	0.7
Reduced PPF (neutral shade)	5.1 (30%)	4.8 (30%)	1.28
Reduced PPF and R:FR (canopy shade)	5.3 (30%)	3.9 (25%)	0.7

^aAveraged daily photosynthetic photon flux (PPF) estimated from instantaneous measurements taken every 15 min from 1 Sept. to 31 Oct. 2008 and 2009.

^bRed to far red light ratio (R:FR) calculated from the wavelengths of 650–670 and 720–740 nm.

Table 2. Color ratings of nontransformed control (NTC), Ax6548, and Ax6549 (*n* = 3) grown in full sun and shade conditions in 2008 and 2009.

Line	2008								2009							
	1 Sept.	11 Sept.	21 Sept.	1 Oct.	11 Oct.	21 Oct.	31 Oct.		1 Sept.	11 Sept.	21 Sept.	1 Oct.	11 Oct.	21 Oct.	31 Oct.	
<i>Full sun^a</i>																
NTC	7.0 ^c c ^y	7.0 c	7.0 c	7.0 c	7.0 c	7.0 c	7.0 c		7.0 c	7.0 c	7.0 c	7.0 c	7.0 c	7.0 c	7.0 c	
Ax6548	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a		9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	
Ax6549	8.0 b	8.0 b	8.0 b	8.0 b	8.0 b	8.0 b	8.0 b		8.0 b	8.0 b	8.0 b	8.0 b	8.0 b	8.0 b	8.0 b	
<i>Reduced R:FR^b</i>																
NTC	7.0 c	7.0 b	7 b	7.0 b	6.7 b	6.0 c	6.0 c		7.0 c	7.0 c	7.0 b	6.3 c	6.7 b	7.0 b	6.0 c	
Ax6548	9.0 a	8.3 a	8.3 a	8.0 a	8.0 a	8.0 a	8.0 a		9.0 a	9.0 a	8.0 a	7.0 b	7.3 b	7.0 b	7.3 b	
Ax6549	8.0 b	8.0 a	8.0 a	8.0 a	8.0 a	8.0 a	8.0 a		8.0 b	8.0 b	8.0 a	8.0 a	8.0 a	8.0 a	8.0 a	
<i>Reduced PPF^c</i>																
NTC	7.0 c	6.0 b	6.0 b	6.0 b	6.0 b	5.7 b	5.7 b		7.0 c	7.0 c	6.0 c	6.0 c	5.7 b	5.0 b	2.7 b	
Ax6548	9.0 a	7.3 a	7.3 a	7.0 a	7.0 a	7.0 a	7.0 a		9.0 a	9.0 a	7.0 b	7.3 b	7.0 a	7.0 a	7.0 a	
Ax6549	8.0 b	7.0 a	7.0 a	7.0 a	7.0 a	7.0 a	6.7 a		8.0 b	8.0 b	8.0 a	7.7 a	7.0 a	7.0 a	7.0 a	
<i>Reduced R:FR and PPF^d</i>																
NTC	7.0 c	6.0 b	6.0 b	5.7 b	5.0 b	5.0 b	5.0 b		7.0 c	7.0 c	6.0 c	5.0 c	5.0 b	5.0 b	1.0 c	
Ax6548	9.0 a	7.0 a	7.0 a	7.0 a	7.3 a	7.0 a	7.0 a		9.0 a	9.0 a	7.0 b	7.0 b	6.7 a	7.0 a	2.7 b	
Ax6549	8.0 b	7.3 a	7.3 a	7.0 a	7.0 a	7.0 a	7.0 a		8.0 b	8.0 b	8.0 a	7.7 a	7.3 a	7.0 a	7.0 a	
LSD	0.49	0.49	0.49	0.49	0.49	0.49	0.49		0.82	0.82	0.82	0.82	0.82	0.82	0.82	

^aColor ratings were based on a 1 to 9 scale with 1 being brown, 5 yellow, and 9 being blue–green; acceptable color 6 or greater.

^bWithin all columns means followed by the same letter are not significantly different at *P* = 0.05.

^cPPF = 16.6 (2008) and 16.5 (2009) (mol photons/m²/d), R:FR = 1.28.

^dPPF = 11.8 and 10.3 (2009), R:FR = 0.7.

^ePPF = 5.1 and 4.8 (2009), R:FR = 1.28.

^fPPF = 5.3 and 3.9 (2009), R:FR = 0.7.

R:FR = red to far red; PPF = photosynthetic photon flux; LSD = least significant difference.

Color. *GA2ox* overexpression resulted in significantly ($P = 0.05$) darker color in both modified plants as compared with the NTC plants despite light treatments (Table 2). This was in agreement with previous studies on transgenic rice (*Oryza sativa*) (Sakamoto et al., 2001), tobacco (*Nicotiana tabacum*) (Biemelt et al., 2004; Schomburg et al., 2003), poplar trees (*Populus tremula* × *Populus alba*) (Busov et al., 2003), *Arabidopsis* (*Arabidopsis thaliana*) (Radi et al., 2006), and bahiagrass (*Paspalum notatum* L.) (Agharkar et al., 2007) where *GA2ox* overexpression resulted in plants with darker green leaves. For both years, the color of NTC plants, Ax6548, and Ax6549 grown in full sun conditions was rated as 7, 9, and 8, respectively. Color of all examined lines was reduced by shade treatments. Under reduced R:FR light treatment, color decreased most rapidly and by the largest amount in Ax6548. In 2008, discoloration of Ax6548 was noted on Day 10 of the experiment, whereas in 2009, this was detected on Day 20 and Day 30. First change in color of NTC plants was noted on Day 50 in 2008 and Day 30 in 2009. Reduced R:FR light treatment did not have an effect on color of Ax6549. Neutral shade and canopy shade had the same effect on color of transgenic plants. In the case of Ax6548 under both shade treatments, discoloration was noted on Day 10 in 2008 and Day 20 in 2009. In 2009, an additional substantial drop in Ax6548 color was noted under canopy shade on Day 60 of the study. During both experiments, color of Ax6549 was less affected. In 2008, a drop in color was noted on Day 10, and in 2009, on Day 40 of the experiment. Color of NTC plants was more affected by canopy shade than neutral shade. In 2008, under both shade treatments, first discoloration was noted on Day 10; however, under canopy shade on Day 40, color dropped again. In 2009, the effect of these treatments on the color of NTC was more severe. In the case of neutral shade, a decrease in color was noted on Day 20, Day 50, and Day 60, resulting in color rated as 3. Color of NTC plants exposed to canopy shade decreased on Days 10 and 30. By Day 60, color of NTC plants was rated as 1 as a result of no living tissue left. No experiments were previously performed to evaluate color of *GA2ox*-overexpressing plants as affected by different shade treatments, and few studies investigated the effect of TE on turf quality under shade. However, Ervin et al. (2004) and Goss et al. (2002) reported that TE applications improved color of creeping bentgrass grown under reduced light conditions.

Coverage. Turfgrass coverage was the highest in both years when grown in full sun and lowest under both canopy and neutral shade treatments (Figs. 1 and 2). On Day 1 of both experiments, coverage of transgenic plants was 5% lower ($P = 0.05$) than that of NTC plants as a result of the slower establishment; however, in the full sun treatment, these differences diminished by the end of the study. All shade treatments caused a more severe decline in turf coverage in 2009, presumably as a result of the increased clipping frequency. By the end of the 2008 study, no significant

Fig. 1. Percent of coverage of nontransformed control (black circles), Ax6548 (open circles), and Ax6549 (black triangles) ($n = 3$) grown in full sun (A), reduced red to far red (R:FR) (B), neutral shade (C), and canopy shade (D) in 2008. Error bars designate SES.

Fig. 2. Percent of coverage of nontransformed control (black circles), Ax6548 (open circles), and Ax6549 (black triangles) ($n = 3$) grown in full sun (A), reduced red to far red (R:FR) (B), neutral shade (C), and canopy shade (D) in 2009. Error bars designate SES.

($P = 0.05$) differences were noted in turfgrass coverage among the genotypes grown under reduced R:FR light treatment. However, it is important to note that NTC plants and transgenic plants differed with respect to the onset and rate of decline. First loss in coverage of NTC and transgenic plants was noted on Day 20 and Day 60 of the experiment, respectively, and led to total loss of 18% of turf in NTC plants and 10% in both transgenic plants. In 2009, a decrease in density in all genotypes under reduced R:FR light was noted on Day 30. Plants varied in the rate of decline with ultimate loss of 43%, 62%, and 10% of density

for NTC, Ax6548, and Ax6549, respectively. During the 2008 experiment, canopy shade had a more detrimental effect on turfgrass coverage compared with neutral shade regardless of the line ($P = 0.05$). By 31 Oct., coverage of NTC, Ax6548, and Ax6549 was decreased by 27%, 23%, and 15% under neutral shade, and 43%, 33%, and 33%, respectively under canopy shade (Figs. 2 and 3). Under both shade treatments, the first significant ($P = 0.05$) decline of NTC plant coverage was noted on Day 20 of the experiment, whereas that of transgenic plants was delayed by 20 d. In 2009, both neutral and canopy shade caused

Fig. 3. Quality of the Ax6548 (A), Ax6549 (B), and nontransformed control (C) grown in full sun, reduced red to far red (R:FR), neutral shade, and canopy shade (from left to right) on Day 60 of the experiment in 2008.

Fig. 4. Quality of the Ax6548 (A), Ax6549 (B), and nontransformed control (C) grown in full sun, reduced red to far red (R:FR), neutral shade, and canopy shade (from left to right) on Day 60 of the experiment in 2009.

similar effects. First loss in turf coverage was noted on Day 30 in all genotypes. However, first significant differences among the lines were noted on Day 50 when Ax6549 had the highest ($P = 0.05$) and NTC lowest ($P = 0.05$) coverage. On Day 60 of the experiment, coverage of NTC, Ax6548, and Ax6549 was 2%, 5%, and 45% under neutral shade and 0%, 1%, and 43% under canopy shade (Figs. 2 and 4). To this end no studies were published that evaluated the effect of *GA2ox* overexpression on turfgrass performance under reduced light conditions. However, Nangle (2008) reported a delay in creeping bentgrass coverage loss with TE applications.

Conclusions

Although shade treatments caused a significant decrease in overall turf quality, Ax6549 maintained significantly higher coverage

compared with the other plants. Vertical growth rate was previously defined as a measure of shade adaptation (Tegg and Lane, 2004). Our results showed that dwarfism induced by *GA2ox* overexpression may contribute to enhanced shade tolerance by slowing down the rate of decline in these plants. However, this was not the case with dwarf creeping bentgrass plants overexpressing *AtBAS1* in which, although the decline in quality was delayed, it was more rapid, resulting in poorer turfgrass quality (Studzinska et al., 2009).

A prior study showed that *GA2ox* overexpression affects not only plant growth, but also morphology, biomass accumulation, and photosynthetic capacity (Biemelt et al., 2004). Given that shade tolerance is a combination of morphological and physiological traits, additional research is needed to elucidate the mechanism of shade tolerance in transgenic bentgrass.

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