THE EFFECT OF ALTERED ASSIMILATE ALLOCATION AND PARTITIONING DUE TO *PCGA2-OXIDASE* OVEREXPRESSION ON THE GROWTH AND PERFORMANCE OF CREEPING BENTGRASS (*AGROSTIS STOLONIFERA* L.) IN FULL SUN AND REDUCED LIGHT

DISSERTATION

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ABSTRACT

Creeping bentgrass (*Agrostis stolonifera* L.) is a species commonly used in high maintenance turf areas where it is often exposed to shade. Shade stress induces harmful physiological and morphological changes in turf plants which, under intense management conditions, ultimately lead to loss of turfgrass coverage. Adaptive responses to light stimuli are mediated, in part, by phytohormones, gibberellins. Suppression of gibberellin levels by overexpression of genes involved in their catabolism has been proven to successfully control plant stature. Further, it was shown to alter plant morphological and photosynthetic characteristics.

The main objective of this study was to determine the effect of runner bean *(Phaseolus coccineus) GA2-oxidase* gene (*PcGA2ox*) overexpression on creeping bentgrass quality and performance in light limited environments, in the perspective of potential changes in assimilate partitioning and allocation caused by the transformation. Studies were performed at The Ohio State University, Columbus, Ohio, USA from September 1 2008 to July 31 2010. Two genetically modified lines, Ax6548 and Ax6549, transformed with *CP4 EPSPS* and the *PcGA2ox* genes and nontransformed control were included in the study.

The purpose of the first experiment was to evaluate overall visual quality of plants grown under different light conditions and low mowing height. Two greenhouse studies

were conducted from September 1 to October 31, in both 2008 and 2009. Lines were grown under 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) conditions. Turf was evaluated visually for color and percent of coverage. *GA2ox* overexpression resulted in superior quality of Ax6549 under all shade treatments by delaying the decline of its color and coverage.

The aim of the second study was photosynthetic characterization of the lines. The greenhouse studies were performed in September-October 2009 and April-May 2010. Plants were subjected to 4 light treatments as described above. Obtained data showed increased (P=0.05) net CO_2 uptake rates and higher maximum quantum yield of photosynthesis in leaves of Ax6549 across all light treatments. Increased CO_2 uptake rates in Ax6549 were most likely due to lower specific leaf area and higher stomatal conductance. Lines did not differ in regards to dark respiration rates and light compensation points despite the light treatment. In conclusion, higher photosynthate supply per leaf area unit and enhanced low light use efficiency were suggested as potential factors associated with the superior quality of Ax6549 under reduced light conditions

The purpose of the last study was to investigate potential differences in assimilate partitioning of the plants. Morphological and growth analysis were performed under controlled environment conditions in July 2010. Plants were subjected to high and reduced irradiance treatments and evaluated every 10 days. *PcGA2ox* overexpression resulted in reduced biomass accumulation in both transgenic plants. Further, Ax6549

plants were characterized by lower growth efficiency and leaf biomass production efficiency. Although this was in contrary to data obtained during photosynthetic characterization, it could be explained by increased self shading in Ax6549 due to greater leaf width and more horizontal leaf orientation. Under high irradiance conditions, Ax6549 invested more assimilate into roots while Ax6548 into leaves. Under reduced irradiance condition, all plants partitioned more biomass into leaves at the expense of root biomass. Transgenic plants invested more assimilate into leaves compared to nontransformed control plants. Morphological data did not help clarify the methods of improved performance of Ax6549 under shaded conditions. Whole canopy photosynthetic measurements performed at different cutting heights are likely needed to further explain the incongruities between photosynthetic and growth analysis data.

To my parents:

Wanda and Wiesław

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Fields of Study

Major Field: Horticulture and Crop Science

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CHAPTER 1

LITERATURE REVIEW

CREEPING BENTGRASS REVIEW

Within the family Poaceae (Gramineae), the genus *Agrostis* (bentgrass) is in the supertribe Poae and tribe Aveneae (Warnke, 2002). The genus, native to Eurasia (Beard, 1973), consists of approximately 200 species (Hitchcock, 1951). For turfgrass use, five species have been adopted including: *Agrostis stolonifera* creeping bentgrass; *Agrostis canina* L. velvet bentgrass; *Agrostis capilaris* L. colonial bentgrass; *Agrostis castellana* L. dryland bentgrass; and *Agrostis gigantean* L. redtop bentgrass (Warnke, 2002).

Agrostis stolonifera is a strict allotetraploid with a genome designation of A2A2A3A3. It is a cool-season grass species known by its fine texture and adaptation to mowing heights as low as 3 mm (Warnke, 2002). Although it is native to Eurasia, it has been distributed throughout the world due to its suitability for use on high quality golf course tees, greens, and fairways.

While creeping bentgrass has excellent cold tolerance, it is susceptible to heat injury (Fry and Huang, 2004), and has poor wear tolerance (Beard, 1973). It tolerates partial shading but grows best in full sunlight (Beard, 1973).

SHADE STUDIES REVIEW

'Light is one of the most important environmental factors, providing plants with both a source of energy and informational signals that control their growth and development' (Lambers et al., 2008). The ability of plants to perceive light intensity, duration, direction, as well as its spectral composition, allow them to control complex morphogenetic processes (Kendrick and Kronenberg, 1986). Light can induce leaf formation, leaf expansion, chloroplast differentiation, and inhibit stem elongation (Brutnell, 2006).

Reduced irradiance poses stress on plants by limiting photosynthesis and ultimately plant growth. To maximize light absorption and energy transfer, plants grown in shade undergo changes on both leaf and canopy level. Shade leaves are characterized by higher levels of chlorophyll per unit of fresh mass, lower chlorophyll a to chlorophyll b ratio, higher levels of chlorophyll in light harvesting complexes, and larger thylakoid grana (Lambers et al., 2008). Leaf anatomy and morphology can also be affected by shade leading to the production of larger but thinner leaves (Corre, 1983; Allard et al., 1991; Wherley et al., 2005). Moreover, shaded plants partition more biomass into vertical growth (e.g., elongated internodes and petioles) at the expense of assimilate allocation to lateral branching and leaf and root biomass (Henry and Aarssen, 1997; Hebert et al., 2001; Huber and Wiggerman, 1997).

Although morphological changes induced by low light may be beneficial in natural ecosystems, they can be ineffective and undesirable traits in high maintenance turfgrass settings. Under shaded conditions, frequent mowing at reduced heights will cause excessive

foliage loss and decrease photosynthetic capacity of plants, that results in poor stand quality. In low light environments, turf will have reduced ability to grow, develop and recover from wear.

The estimated area of turf grown under shade conditions in the USA is approximately 25% (Beard, 1973). Therefore growing grass in restricted light environments is one of the biggest concerns for all turfgrass managers. Beyond tree removal, which very often meets a resistance from golf players, common management practices such as raising the mowing height, reducing nitrogen fertilization, overseeding, and reducing traffic can diminish the detrimental effect of shade.

Adaptive changes in plants due to alterations in light quality, quantity and duration are mediated by gibberellins (GA) (Hadden and Kamiya, 1997; Sponsel and Hadden, 2004). Endogenous GAs are plant hormones that are involved in plant developmental processes including the promotion of shoot elongation. GA levels can be suppressed by the use of synthetic growth regulators or genetic manipulation of constituents involved in the signaling and biosynthetic pathway. Among GA inhibiting growth regulators used on turf, trinexapac-ethyl (TE) has been the most widely used to reduce clipping yields and improve turf quality under low light conditions (Goss et al, 2002; Steinke and Stier, 2003; Gardner and Wherley, 2005). TE, a cyclohexanedione, reduces cell elongation in vegetative tissue by blocking gibberellic acid (GA) biosynthesis (Heckman et al., 2002). TE competitively inhibits the conversion of GA_{20} to GA_1 by 3-ß-hydroxylase, markedly reducing leaf cell elongation (Adams et al. 1992) but not cell division (Ervin and Koski, 2001).

Frequent growth regulator applications can be costly and may raise environmental concerns. Biotechnological manipulation of GA levels provides an alternative approach and has been successfully utilized in plant stature control (Sacamoto et al., 2001; Schomburg et al., 2003; Busov et al., 2003; Biemelt et al., 2004; Radi et al., 2006; Agharkar et al., 2007).

GIBBERELLIN REVIEW

Gibberellins are a large group of naturally occurring compounds with 136 different forms currently known from higher plants, fungi and bacteria. However, only a few of GAs have biological activity including GA_1, GA_3, GA_4, GA_5, GA_6, and GA_7.

Gibberellins were first isolated by Japanese scientists in 1930s from the pathogenic fungus *Gibberella fujikuroi* (Phinney, 1983). The GAs secreted by fungi caused extensive growth of infected plants thus from the time of their discovery, they were known to be effective in promoting stem elongation (Sponsel and Hedden, 2004).

Although, they were originally discovered as the cause of disease symptoms that resulted in internode elongation, endogenous GAs influence a large number of developmental processes. The gibberellin hormones act throughout the life cycle of plants, influencing seed germination, flower induction, pollen development, and fruit growth (Sponsel and Hedden, 2004). Furthermore, they mediate environmental stimuli, which modify the flux through the GA-biosynthetic pathway; therefore regulation of GA biosynthesis is of fundamental importance to plant development and its adaptation to the environment (Hadden and Kamiya, 1997).

Gibberellin biosynthesis and catabolism

Gibberellins are tetracyclic diterpenoid acids with structures based on the *ent*-gibberellane carbon skeleton (Sponsel and Hedden, 2004). They are synthesized from geranylgeranyl diphosphate (GGPP) *via* isopenthyl diphosphate in young tissue of the shoot and developing seed (Sponsel and Hedden, 2004). The GAs biosynthetic pathway can be divided into three parts, according to their subcellular compartmentalization and enzymes involved (Pimenta and Lange, 2006). The formation of GA is initiated in the plastids by cyclization of GGPP to *ent*-kaurene in two-step process catalyzed by *ent*-copatyl diphosphate synthase and *ent*-kaurene synthase (Lange, 1998). Subsequently, ent-kaurene is oxidized in six steps to GA_{12} and its 13-hydroxylated analog GA_{53} by the action of cytochrome P450 monooxygenases at the endoplasmic reticulum (Lange, 1997; Hedden and Proebsting, 1999). Finally, in the cytosol, GA_{12} and GA_{53} are converted by 2-oxoglutarate-dependent dioxygenases (GA20-oxidases and GA3-oxidases) to bioactive products, GA_4 and GA_1, respectively (Sponsel and Hedden, 2004). Bioactive GA_1 and GA_4 can be then converted by 2β-hydroxylation catalyzed by GA2-oxidases (GA2ox) to inactive GA_8 and GA_{34} (Hedden and Proebsting, 1999).

GA2- oxidazes review

GA2ox are soluble 2-oxoglutarate-dependent dioxygenases that are responsible for the irreversible deactivation of GAs by 2β-hydroxylation (Sponsel and Hedden, 2004). Most 2-oxidases are specific for C19-GAs and accept the 3β-hydroxy bioactive GAs and their non-3β-hydroxylated precursors as substrates (Sponsel and Hedden, 2004).

GA2ox are encoded by multigene families, members of which differ in their positional and temporal patterns of expression (Thomas et al., 1999)

Genes encoding for GA2ox were isolated from runner bean and *Arabidopsis* (Thomas et al., 1999), garden pea (Lester et al., 1999, Martin et al., 1999), rice (Sacamoto et al., 2001; Sacamoto et al. 2003), pumpkin (Frise et al., 2003), and poplar (Busov et al., 2003). In these assays GA2ox caused conversion of bioactive GAs, GA_1 and GA_4 and their immediate precursors GA_{20} and GA_9 to the corresponding 2β-hydroxy products.

Ectopic expression 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 (Sacamoto et al., 2001). Similar phenotype was obtained by expressing *GA2ox* in transgenic tobacco plants (Schomburg et al., 2003; Bimelt et al., 2004), poplar trees (Busov et al., 2003), and *Arabidopsis* (Radi et al., 2006). Overexpression of *AtGA2ox1* in bahiagrass (*Paspalum notatum* L.) produced a semi-dwarf phenotype with increased tillering, delayed flowering, and shorter inflorescence, thus enhancing its overall quality (Agharkar et al., 2007).

It was shown that *GA2ox* expression not only affects plant growth but also morphology, biomass accumulation, and photosynthetic capacity. Examination of leaves and stems of tobacco plants transformed with *AtGA2ox* revealed a thicker spongy parenchyma layer, an extra palisade layer; and increased number of pith cells with decreased number of xylem cells compared to wild type (Bimelt et al., 2004). Additionally, down regulation of GA biosynthesis decreased biomass accumulation and increased fresh to dry matter ratio which was due to decreased lignin deposition (Bimelt

et al., 2004). Single leaf photosynthesis measurements performed on the same plants have shown increased rates of CO_2 uptake and higher maximum quantum yield of photosystem II (PSII) compared to wild type plants concomitant with increased chlorophyll levels.

Light regulation of GA Biosynthesis and Catabolism

GAs are intermediaries for a number of environmental signals, which may induce changes in GA concentration and/or sensitivity (Sponsel and Hadden, 2004). Light quality, quantity and duration can all influence the rate of GA biosynthesis and catabolism through their effects on expression of specific genes (Sponsel and Hadden, 2004). Developmental processes that are regulated by light induced changes in GA biosynthesis are: seed germination, de-etiolation, and flowering induction.

Red light induced expression of GA3ox genes in *Arabidopsis* (Yamaguchi et al., 1998) and lettuce (Toyomasu, 1998) seeds. In etiolated pea seedlings, light caused phytochrome mediated down regulation of the *GA3ox1* and up regulation of *GA2ox* (Reid et al., 2002). Subjecting pea seedlings to low irradiance enhanced production of C19-GA showing that not only quality of light but also quantity may influence GA content in the plants (Gawronska et al., 1995). GAs also have been shown to act as secondary messengers in processes induced by long photoperiods such as flowering in long-day rosette plants, the breaking of bud dormancy in woody plants, and the induction of stolons rather than tubers in potato. Exposure of Arabidopsis (Kamiya and Garcia-Martinez, 1999) and spinach (Wu et al., 1996) to long days increased GA20ox expression in these plants, leading to rapid stem elongation and accelerated flowering. Potato

tuberization is also prevented in long days by higher activity of GA20-oxidase which results in increased GAs concentration (Carrera, 1999).

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CHAPTER 2

THE EFFECT OF *PCGA2OX* OVEREXPESSION ON CREEPING BENTGRASS (*AGROSTIS STOLONIFERA* L.): PERFORMANCE UNDER VARIOUS LIGHT ENVIRONMENTS

ABSTRACT

Turf grown in shade exhibits increased stem elongation and becomes weakened. Dwarfism could improve turfgrass quality and reduce management cost. 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 September 1 to October 31, in both 2008 and 2009. Two experimental lines, Ax6548 and Ax6549, transformed with *CP4 EPSPS* and *PcGA2ox* gene; and a nontransformed control (NTC) were subjected to four light environments: full sun, reduced R:FR light, canopy shade (reduced photosynthetic photon flux and red: far red ratio), and neutral shade (reduced PPF). Turf was evaluated every 10 days for color and percent of coverage. *GA2ox* overexpression resulted in darker green color in both transgenic lines under all light treatments as compared to 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.

INTRODUCTION

Creeping bentgrass (*Agrostis stolonifera* L.) is a turfgrass species highly suitable for use on golf course tees, greens, and fairways. Due to 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).

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 its spectral composition which act in concert to determine turf performance. Reduction of photosynthetic photon flux (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 (Dudeck and Peacock, 1992; Bell and Danneberger, 1999; Koh et al. 2003; Wherley et al., 2005). Moreover turfgrasses grown in low PPF environments were characterized by longer, thinner, and more succulent leaves (Wilkinson and Beard, 1974; Allard et. al. 1991; Wherley et al., 2005). Alteration in spectral composition and specifically reduced R:FR light ratio further contributes to aforementioned morphological changes (Casal et al. 1990; Dudeck and

Peacok, 1992; Frank and Hoffman, 1994; Wherley et al., 2005). However, Wherely et al. (2005) reported that leaves of plants grown under low PPF but high R:FR ratio were wider compared to those grown under low PPF and low R:FR light environment.

Under golf course conditions, shaded creeping bentgrass greens are maintained by frequent mowing at reduced heights that causes excessive foliage loss. 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) (Hadden and Kamiya, 1997; Sponsel and Hadden, 2004). GAs are phytohormones that are involved in many developmental processes including stem elongation (Davis, 2007). They act by induction of 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 constituents involved in signaling and biosynthetic pathway.

Among GA inhibiting growth regulators, trinexapac-ethyl (TE) suppresses vertical growth and improves turf overall quality under low light conditions (Goss et al, 2002; Steinke and Stier, 2003). TE competitively inhibits the conversion of GA_{20} to GA_1 by 3-ß-hydroxylase, reducing leaf cell elongation (Adams et al. 1992) but not cell division (Ervin and Koski, 2001). However frequent applications can be expensive and may raise environmental concerns.

In plants, inactivation of bioactive gibberellins GA_1 and GA_4 is ensured by GA2-oxidases (GA2ox) that catalyze their 2β-hydroxylation yielding GA_8 and GA_{34} (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 (Sacamoto et al., 2001.). A similar phenotype was obtained by expressing *GA2ox* in transgenic tobacco plants (Schomburg et al., 2003; Bimelt et al., 2004), poplar trees (Busov et al., 2003), and *Arabidopsis* (Radi et al., 2006). Overexpression of *AtGA2ox1* in bahiagrass (*Paspalum notatum* L.) produced a semi-dwarf 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 et al., 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 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 et al. (2006), two superior lines were selected for further research, Ax6548 and Ax6549, transformed with *CP4 EPSPS* and *PcGA2ox* genes. Nontransformed plants (NTC) 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, Marysville, OH) 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 days and in 2009 every 5 days.

Plants were fertilized at the rate of 0.2 kg of N per 92.9 m^2, using 100 ppm solution of 20N-10P-20K fertilizer (Scotts/Sierra, USA). Monthly applications of chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) and iprodione (3-(3,5-dichlorophenyl) -N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxyamide) 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 insecticides were performed to prevent pest occurrence. Plants were irrigated to replenish water loss through evapotranspiration.

Treatments and Experimental Design

Research was conducted at The Ohio State University, Columbus, OH. Two greenhouse experiments were performed from September 1 to October 31, 2008 and 2009. Both years, plants were randomly assigned to four irradiance treatments: full sun (control), photoselective 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 2.1). The experimental design was a split-plot with 3 replicates with irradiance treatments as a main plots and turfgrass genotypes as subplots.

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

Data collection

Turf was examined every 10 days, starting from day 1, for color and coverage. Color of living tissue was rated visually, by the primary author only, using a 1-9 scale with 1 being brown, 5 yellow, and 9 blue-green. Pot coverage was rated as a percentage of potential coverage.

Statistical analysis

To test the effect of light, gene transformation, and their interaction, data from both experiments were analyzed by analysis of variance (ANOVA) for split plot design, using PROC MIXED (SAS Institute, Cary, NC). Standard error of means (SEM) was used to calculate least significant differences (LSD) at P=0.05 for means separation.

RESULTS AND DISCUSSION

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

Color

GA2ox overexpression resulted in significantly (P=0.05) darker color in both modified plants as compared to the NTC plants despite light treatments (Table 2.2 and 2.3; Fig. 2.1, 2.3, 2.5, and 2.6). This was in agreement with previous studies on transgenic rice (Sacamoto et al., 2001), tobacco (Schomburg et al., 2003; Bimelt et al., 2004), poplar trees (Busov et al., 2003), *Arabidopsis* (Radi et al., 2006) and bahiagrass (Agharkar et al., 2007) where *GA2ox* overexpression resulted in plants with darker green leaves. For both years, the color of NTC plants, Ax6548, Ax6549 grown in full sun conditions was rated as 7, 9, and 8, respectively. Color of all examined lines was affected

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 while 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 case of Ax6548 under both shade treatments discoloration was noted on day 10 in 2008 and day 20 in 2009. In 2009, 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, drop in color rating 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 rating dropped again. In 2009, the effect of these treatments on the color of NTC was more severe. In case of neutral shade decrease in color was noted on day 20, 50 and day 60, resulting in color rated as 3. Color of NTC plants exposed to canopy shade decreased twice - on day 10 and 30. By day 60, it was rated as 1 due to no living tissue left. Although no experiments were previously performed to evaluate color of *GA2ox* overexpressing plants as affected by different shade treatments, few studies investigated the effect of TE on turf quality under shade. Goss et al. (2002) and Ervin et al. (2004) 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 (Table 2.2 and 2.3; Fig. 2.2 and 2.4). On day one of both experiments, coverage of transgenic plants was 5 % lower (P=0.05) than that of NTC plants due to the slower establishment; however, in full sun treatment, these differences diminished by the end of the study. All shade treatments caused more severe decline in turf coverage in 2009, presumably due to the increased clipping frequency. By the end of the 2008 study, no significant (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 in 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 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 to neutral shade regardless of the line (P=0.05). By Oct 31, coverage of NTC, Ax6548 and Ax6549 was decreased by 27, 23, and 15 % under neutral shade, and 43, 33, and 33 % under canopy shade (Fig. 2.6). Under both shade treatments the first significant (P=0.05) decline of NTC plants coverage was noted on day 20 of the experiment while that of transgenic plants was delayed by 20 days. In 2009, both neutral and canopy shade caused 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 highest (P=0.05) and NTC plant lowest (P=0.05) coverage. On day 60 of the experiment coverage of NTC, Ax6548 and Ax6549 was rated as 2, 5, and 45 % under neutral shade, and 0, 1, and 43 % under canopy shade (Fig. 2.7). To this end no studies were published that evaluated the effect of *GA2ox* overexpression on turfgrass performance under reduced light conditions. However, Nangle et al. (2008) reported a delay in creeping bentgrass coverage loss with TE applications.

CONCLUSIONS

Although shade treatments caused significant decrease in overall turf quality, Ax6549 maintained significantly higher coverage compared to the other plants. Vertical growth rate was previously defined as a measure of shade adaptation (Tegg & 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* where, although the decline in quality was delayed, it was more rapid, resulting in poorer turfgrass quality (Studzinska et al., 2009). Further research is needed to evaluate physiological and morphological factors that may underlay shade tolerance in these plants.

Light treatment	**PPF** [mol photons m^{-2} d^{-1}] (% of full sun value)		**R:FR**
	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 & R:FR (Canopy shade)	5.3 (30%)	3.9 (25 %)	0.7

†Averaged daily PPF estimated from instantaneous measurements taken every 15 min from September 1 to October 31, 2008 and 2009.

‡ Red to far red light ratio calculated from the wavelengths of 650-670 and 720-740 nm.

Table 2.1: Light conditions for September-October 2008 and 2009.

	Color ratings†							Density ratings‡						
Line	**1-Sep**	**11-Sep**	**21-Sep**	**1-Sep**	**11-Oct**	**21-Oct**	**31-Oct**	**1-Sep**	**11-Sep**	**21-Sep**	**1-Sep**	**11-Oct**	**21-Oct**	**31-Oct**
							Full sun¶							
NTC	7c§	7c	7c	7c	7c	7c	7c	100a	100a	98a	100a	100a	100a	96a
Ax6548	9a	9a	9a	9a	9a	9a	9a	95b	95b	95a	99a	100a	100a	100a
Ax6549	8b	8b	8b	8b	8b	8b	8b	95b	95b	95a	95b	97a	100a	100a
							Reduced R:FR#							
NTC	7c	7b	7b	7b	7b	6c	6c	100a	100a	95a	96a	96a	95a	82a
Ax6548	9a	8a	8a	8a	8a	8a	8a	95b	95b	95a	95a	96a	95a	85a
Ax6549	8b	8a	8a	8a	8a	8a	8a	95b	95b	97a	95a	97a	95a	85a
							Reduced PPF††							
NTC	7c	6b	6b	6b	6b	6b	6b	100a	100a	90a	93a	83a	78ab	73b
Ax6548	9a	7a	7a	7a	7a	7a	7a	95b	95b	91a	91a	84a	77b	77ab
Ax6549	8b	7a	7a	7a	7a	7a	7a	95b	95b	90a	91a	83a	82a	80a
							Reducer R:FR and PPF§§							
NTC	7c	6b	6b	6b	5b	5b	5b	100a	100a	92a	87b	80b	75b	57b
Ax6548	9a	7a	7a	7a	7a	7a	7a	95b	95b	94b	94a	87a	80a	67a
Ax6549	8b	7a	7a	7a	7a	7a	7a	95b	97ab	95b	92a	87a	75b	67a

†Color ratings were based on 1-9 scale with 1 being light green and 9 being dark green; acceptable color ≥6.
‡Coverage was rated as a % of potential coverage.
§ Within all columns means followed by the same letter are not significantly different at *P*=0.05.
¶ PPF=16.6 [mol photons m^{-2} d^{-1}] , R:FR=1.28.
# PPF=11.8, R:FR=0.7.
†† PPF=5.1, R:FR=1.28.
§§ PPF=5.3, R:FR=0.7.

Table 2.2: Color and coverage ratings of nontransformed control (NTC), Ax6548, and Ax6549 (n=3) grown in full sun, and shade conditions in 2008.

	Color ratings†							Density ratings‡						
Line	**1-Sep**	**11-Sep**	**21-Sep**	**1-Sep**	**11-Oct**	**21-Oct**	**31-Oct**	**1-Sep**	**11-Sep**	**21-Sep**	**1-Sep**	**11-Oct**	**21-Oct**	**31-Oct**
							Full sun¶							
NTC	7c§	7c	7c	7c	7c	7c	7c	100a	100a	100a	95a	90a	90a	95a
Ax6548	9a	9a	9a	9a	9a	9a	9a	95a	95a	95a	90a	93a	93a	95a
Ax6549	8b	8b	8b	8b	8b	8b	8b	95a	95a	95a	90a	93a	93a	93a
							Reduced R:FR#							
NTC	7c	7c	7b	6c	7b	7b	6c	100a	100a	100a	90a	87a	68b	57b
Ax6548	9a	9a	8a	7b	7b	7b	7b	95a	95a	95a	80b	80a	63b	38c
Ax6549	8b	8b	8a	8a	8a	8a	8a	95a	95a	95a	87ab	87a	85a	85a
							Reduced PPF††							
NTC	7c	7c	6c	6c	6b	5b	3b	100a	100a	100a	90a	70a	20c	2b
Ax6548	9a	9a	7b	7b	7a	7a	7a	95a	95a	95a	78b	68a	40b	5b
Ax6549	8b	8b	8a	8a	7a	7a	7a	95a	95a	95a	78b	72a	70a	45a
							Reducer R:FR and PPF§§							
NTC	7c	7c	6c	5c	5b	5b	1c	100a	100a	100a	85a	68a	8c	0b
Ax6548	9a	9a	7b	7b	7a	7a	3b	95a	95a	95a	80a	68a	27b	1b
Ax6549	8b	8b	8a	8a	7a	7a	7a	95a	95a	95a	77a	75a	65a	43a

†Color ratings were based on 1-9 scale with 1 being light green and 9 being dark green; acceptable color ≥6.
‡Coverage was rated as a % of potential coverage.
§Within all columns means followed by the same letter are not significantly different at *P*=0.05.
¶ PPF=16.5 [mol photons m^{-2} d^{-1}] , R:FR=1.28.
# PPF=10.3, R:FR=0.7.
†† PPF=4.8, R:FR=1.28.
§§ PPF=3.9, R:FR=0.7.

Table 2.3: Color and coverage ratings of nontransformed control (NTC), Ax6548, and Ax6549 (n=3) grown in full sun, and shade conditions in 2009.

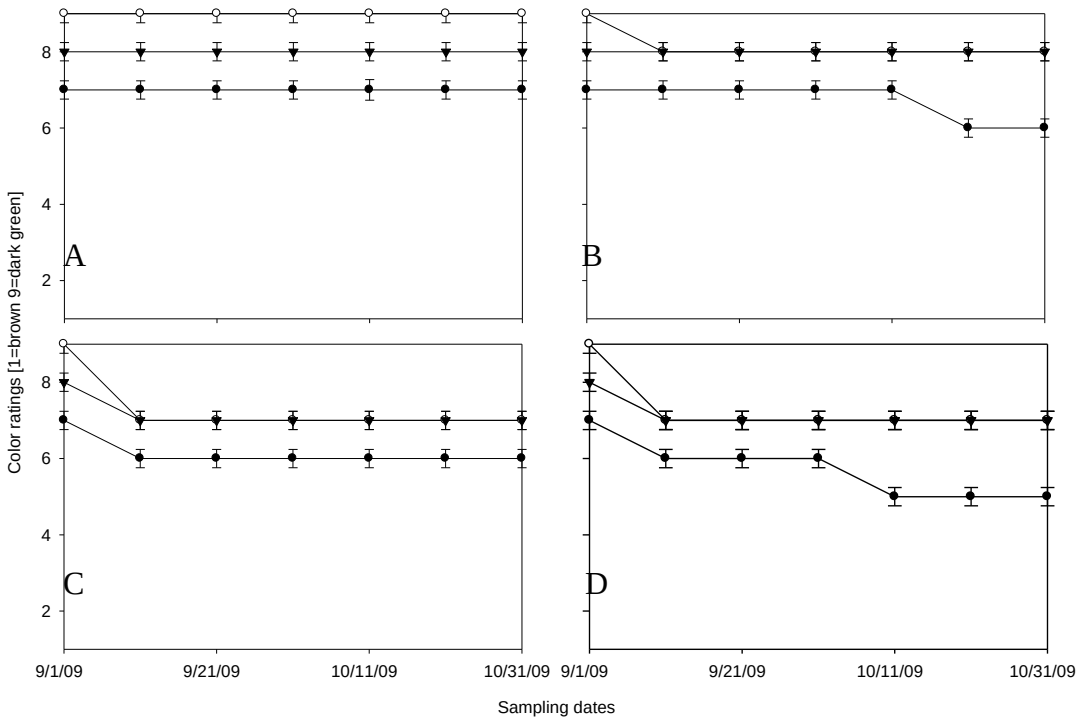

Fig. 2.1: Color ratings of nontransformed control (black circles), Ax6548 (white circles), and Ax6549 (black triangles) (n=3) grown in full sun (A), reduced R:FR (B), neutral shade (C), and canopy shade (D) in 2008. Ratings are based on 1-9 scale; 1=brown color, 9=dark green color; acceptable turf color≥6. Error bars designate standard errors.

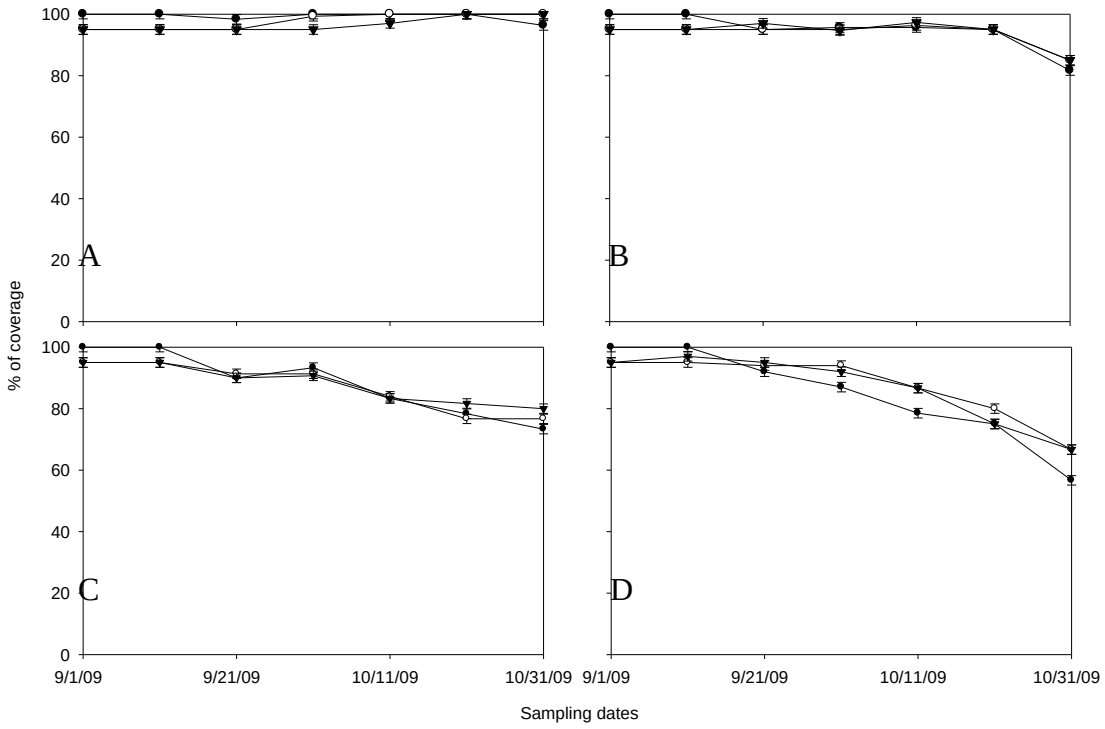

Fig. 2.2: Percent of coverage of nontransformed control (black circles), Ax6548 (white circles), and Ax6549 (black triangles) (n=3) grown in full sun (A), reduced R:FR light (B), neutral shade (C), and canopy shade (D) in 2008. Error bars designate standard errors.

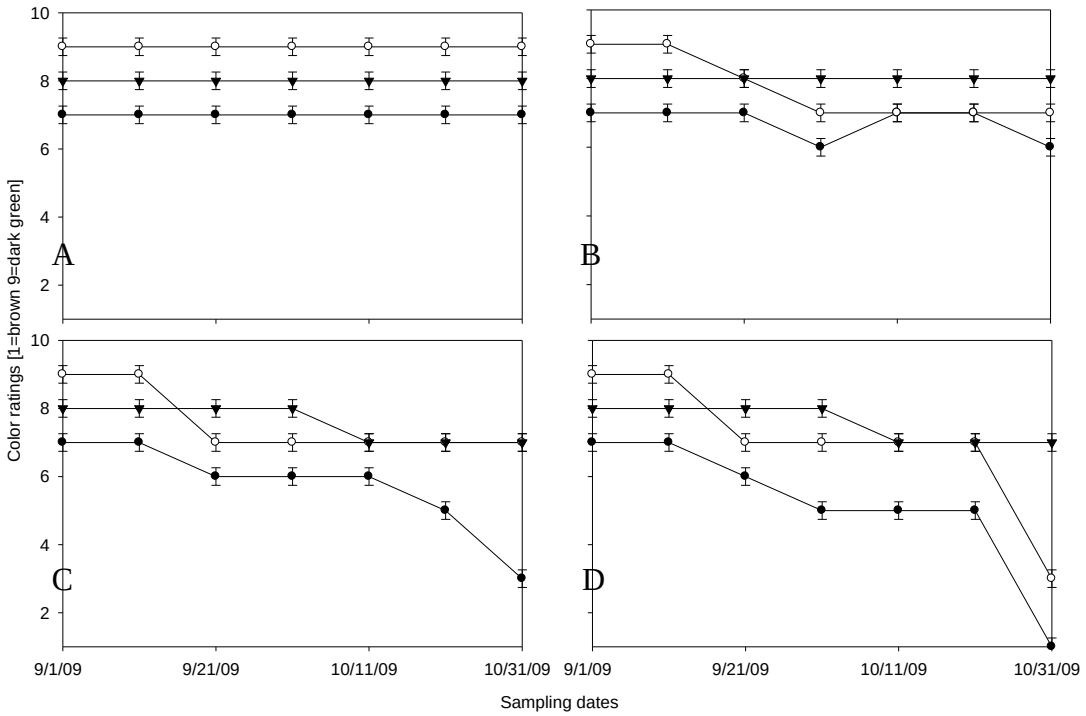

Fig. 2.3: Color ratings of nontransformed control (black circles), Ax6548 (white circles), and Ax6549 (black triangles) (n=3) grown in full sun (A), reduced R:FR (B), neutral shade (C), and canopy shade (D) in 2009. Ratings are based on 1-9 scale; 1=brown color, 9=dark green color; acceptable turf color≥6. Error bars designate standard errors.

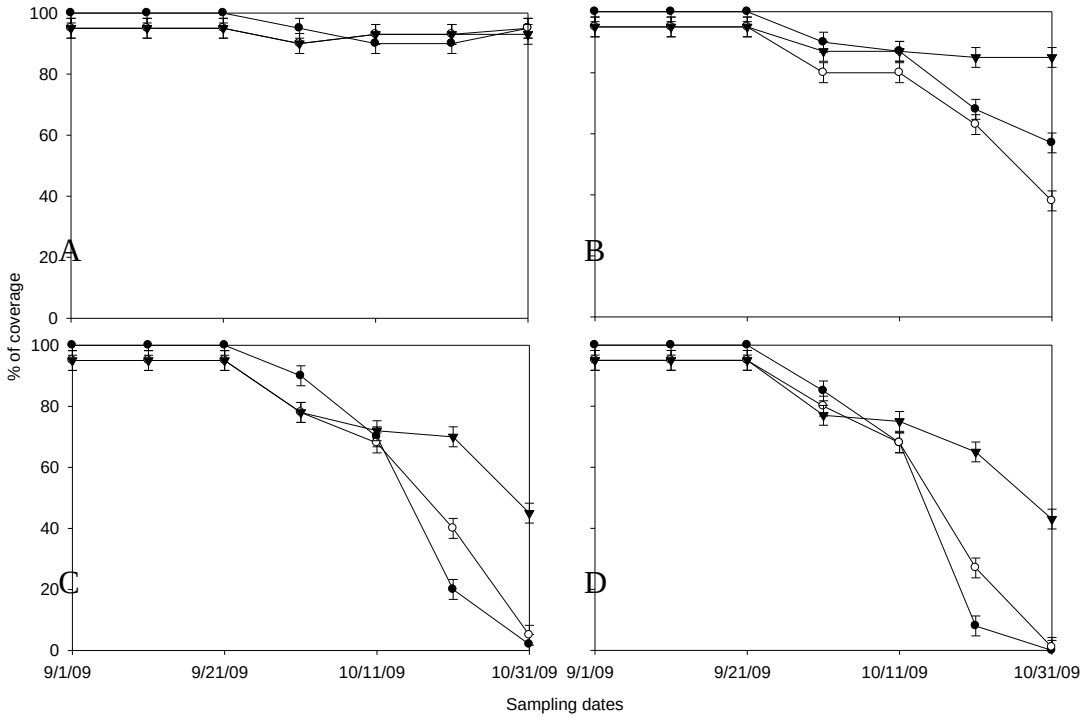

Fig. 2.4: Percent of coverage of nontransformed control (black circles), Ax6548 (white circles), and Ax6549 (black triangles) (n=3) grown in full sun (A), reduced R:FR light (B), neutral shade (C), and canopy shade (D) in 2009. Error bars designate standard errors.

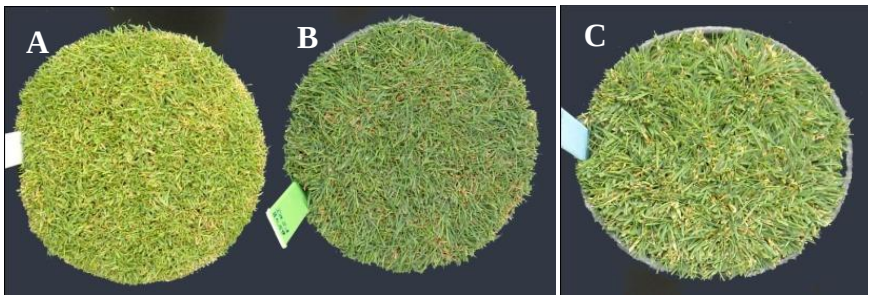

Fig. 2.5: Quality of nontransformed control (A), Ax6548 (B), and Ax6549 (C) at the day 1 of the experiment.

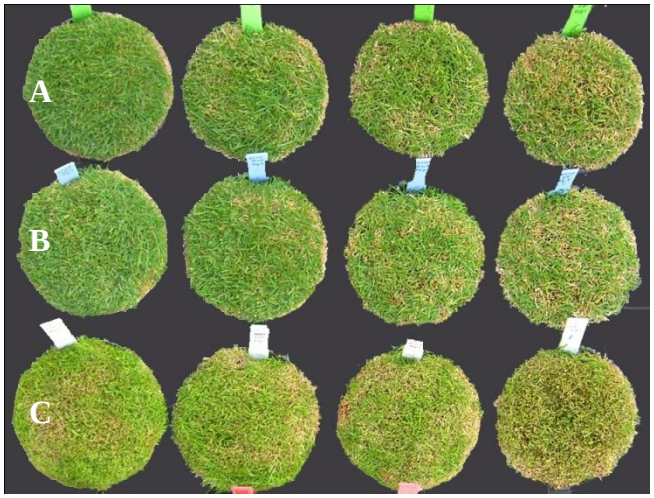

Fig. 2.6: Quality of the Ax6548 (A), Ax6549 (B), and nontransformed control (C) grown in full sun, reduced R:FR light, neutral shade, and canopy shade (from left to right) on day 60 of the experiment in 2008

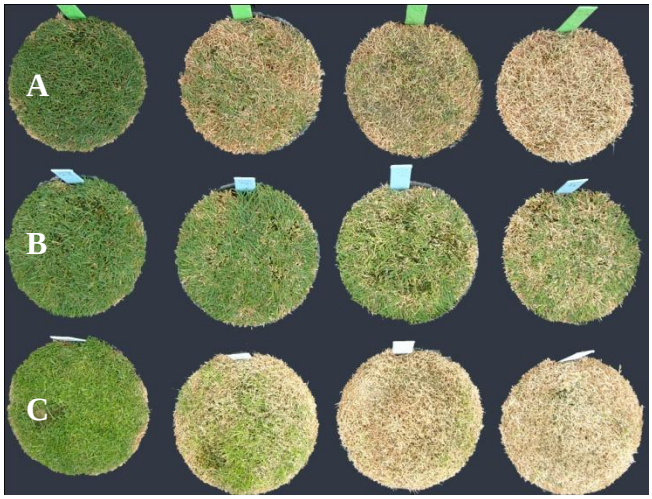

Fig. 2.7: Quality of the Ax6548 (A), Ax6549 (B), and nontransformed control (C) grown in full sun, reduced R:FR light, neutral shade, and canopy shade (from left to right) on day 60 of the experiment in 2009.

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CHAPTER 3

THE EFFECT OF *PCGA2OX* OVEREXPESSION ON PHOTOSYNTHETIC CHARACTERISTICS OF CREEPING BENTGRASS (*AGROSTIS STOLONIFERA* L.) UNDER VARIOUS LIGHT ENVIRONMENTS

ABSTRACT

Gibberellins are phytohormones that mediate environmental stimuli and thus are important for plant adaptive responses. The purpose of this study was to assess physiological factors that may underlay improved shade tolerance in creeping bentgrass Ax6549 line with *PcGA2ox* overexpression. The greenhouse studies were performed at The Ohio State University in September-October 2009 and April-May 2010. Two genetically modified lines, Ax6548 and Ax6549, transformed with *CP4 EPSPS* and the *PcGA2ox* genes, and nontransformed control were grown in full sun, under blue polyethylene film (reduced R:FR), black shade cloth (reduced PPF), and blue polyethylene film with black shade cloth (reduced R:FR and PPF). Photosynthetic characterization showed substantial increase (P=0.05) in net CO_2 uptake rates in leaves of Ax6549 across all light treatments. Further, these plants were characterized by higher maximum quantum yield of photosynthesis. No significant differences (P=0.05) were found among the lines in regards to dark respiration rates and light compensation points

despite the light treatment. In conclusion, higher photosynthate supply per leaf area unit and enhanced low light use efficiency were suggested as potential factors associated with superior quality of Ax6549 under reduced light conditions.

INTRODUCTION

Shade tolerance is defined as a set of morphological and physiological traits that enable plants to grow in a low light environment. Plants grown in shade are characterized by lower leaf light compensation points, lower respiration rates, and lower maximal photosynthetic rates as compared to high irradiance grown plants (Ehleringer, 2006). In order to maintain positive carbon balance and survive in light scarce environment, sun plants like turfgrasses require acclimation.

Several studies had investigated the relationship between turfgrass photosynthetic properties and their overall performance in low light (Wilkinson et al., 1975; Winstead and Ward, 1974; Jiang et al., 2004). Winstead and Ward (1974) compared photosynthetic responses of 'Tiflawn' bermudagrass (*Cynodon dactylon* L.) and St. Augustinegrass (*Stenotaphrum secundatum*), considered as shade tolerant species, to reduced light. Shade treatment resulted in reduced net photosynthetic rates and respiration rates in bermudagrass, but did not affect these parameters in St. Augustinegrass. A similar experiment performed on bermudagrasses and seashore paspalums found that the most shade tolerant paspalum variety, 'Sea Isle 1', experienced a smaller reduction in net photosynthetic rate compared to the least shade tolerant bermudagrass 'TifEagle' (Jiang et al., 2004). Photosynthetic comparisons between shade grown cool season turfgrasses

‘Merion’ Kentucky bluegrass (*Poa pratensis* L.) and ‘Pennlawn’ red fescue (*Festuca rubra* L.) found a similar response for both species regarding net photosynthetic rate, light saturation levels, and light compensation points (Wilkinson at al., 1975). However as opposed to Marion, Pennlawn’s respiration rates were reduced in shade which could be associated with its ability to provide better quality turf in low light environment.

It was previously found that photosynthetic characteristics of plants can be influenced by gibberellins (GA). GAs are naturally occurring plant hormones that influence seed germination, stem elongation, leaf expansion, flower induction, pollen development, and fruit growth (Davis, 1995). They mediate environmental stimuli such as light quality, quantity, and duration; therefore regulation of GA biosynthesis is of fundamental importance to plant development and its adaptation to the environment (Hedden and Kamiya, 1997; Sponsel and Hadden, 2004).

Several studies have been performed to investigate the effect of genetic regulation of GA levels on plant photosynthetic properties; however, they yielded contradictory results. Single leaf photosynthesis measurements revealed lower photosynthetic CO_2 uptake rates in tobacco plants transformed with *GA20ox* (encoding GA biosynthetic enzyme) and increased photosynthetic activity at nearly saturating light intensity in plants transformed with *AtGA2ox* (encoding a GA catabolic enzyme) as compared to wild type (Biemelt, 2004). Furthermore, maximum quantum yield was increased in GA deficient plants and remained unaffected in plants with elevated GA content. In contrast, experiments performed on transgenic Carrizo citrange (*Citrus sinensis* × *Poncirus trifoliata*) plants overexpressing *CcGA20ox1* showed extensive up-regulation of genes

involved in photosynthesis with concomitant increase in net photosynthetic rate (Huerta et al., 2008). Increased photosynthetic capacity of these plants was related by the authors with more compact mesophyll tissue (Faogoaga at al.; 2007, Huerta et al., 2008).

Similarly, the effect of exogenous GA application is inconsistent with some studies reporting that GA_3 may increase (Hayat et al., 2001; Yuan and Xu, 2001; Ashraf at al., 2002), decrease (Dijkstra et al., 1990), or not affect (Cramer et al., 1995; Huerta et al., 2008) photosynthetic capacity of the plants.

Creeping bentgrass is commonly used in high maintenance turf areas, where it is often exposed to reduced light conditions. Depending on the cultivar, it may tolerate partial shading but grows best in full sunlight (Beard, 1973). To this end transgenic creeping bentgrass plants expressing runner bean (*Phaseolus coccineus*) *GA2- oxidase* gene (*PcGA2ox)* were developed. These lines are characterized by dwarf phenotype and delayed decrease in overall stand quality when grown under reduced irradiance levels. In this study I investigated the physiological factors that may underlie improved performance of a modified plant, Ax6549, in shade. The goals were to: (1) examine the effect of *PcGA2ox* overexpression on photosynthetic properties of creeping bentgrass; (2) examine the photosynthetic responses of a nontransformed control and transgenic lines to low light treatments; and (3) characterize leaf morphological and anatomical traits underlying physiological responses.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Yan et al. (2006) identified two superior transgenic creeping bentgrass (*Agrostis stolonifera* L.) lines Ax6548 and Ax6549, transformed with *CP4 EPSPS* and *PcGA2ox* genes. They were selected for physiological and anatomical characterization. A nontransformed control (NTC) selected from 'Crenshaw' variety was included. Plants were propagated vegetatively in 30 cm pots, filled with pure sand as a growing medium, and established for 6 months before subjecting to the light treatments. Plants were maintained at 15 cm height and fertilized at the rate of 0.2 kg of N per 92.9 m^2, using 100 ppm solution of 20N-10P-20K fertilizer (Scotts/Sierra, USA). Fungicides and insecticides were applied when needed to prevent diseases and insects. During the study applications of chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) and iprodione (3-(3,5-dichloro-phenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidine-carboxy-amide) 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-phenoxy-phenyl)methyl 3-(2,2-dichloroethenyl), and bifenthrin insecticides were performed. Irrigation was applied to prevent wilt.

Treatments and Experimental Design

Research was conducted at The Ohio State University, Columbus, OH. In March 2009, four irradiance treatments were imposed: full sun (control), blue polyethylene film (reduced red light to far red light ratio (R:FR)), black shade cloth (neutral shade), and

blue polyethylene film with black shade cloth (simulated canopy shade) (Table 1.). The experiment was arranged as split-plot with 3 replicates where irradiance treatments served as a main plots and creeping bentgrass lines as subplots. Data were collected in September-October, 2009 and April-May, 2010.

Measurements of photosynthetic photon flux (PPF) were taken every 15 minutes using cosine corrected photosynthetically active radiation (PAR) light sensors (Spectrum Technologies, Plainfield, IL). Dataloggers (WatchDog 225; Spectrum technologies, Plainfield, IL) were used to monitor air temperature and relative humidity. Spectral distribution was measured in the range of 400 nm to 800 nm, and red to far red light ratio was calculated from the wavelengths of 650-670 and 720-740 nm (Brutnell, 2006) utilizing a LI-1800 spectroradiometer (LI-COR Bioscience, Lincoln, NE).

Photosynthetic characterization

CO_2 uptake rates were measured using LCi Portable Photosynthesis System (ADC BioScientific Lcd, England). The measurements were carried out in an open system configuration using broad plant leaf chamber (PLC) with the full leaf area of 625 mm^2. Chamber temperature, PAR at the window, PAR incident on the leaf surface, and atmospheric pressure were recorded.

To take the measurements, plants were transferred from the greenhouse to controlled environment chambers to assure uniform light and temperature levels. Prior to the measurements plants were dark incubated for 60 minutes. Gas exchange was measured at light intensity increments of 50, 100, 200, 400, and 600 μmols m^2 s^{-1} of

PAR, constant temperature 20 ± 2 ^{o} C and average CO_2 concentration of 410 vpm. In each experiment, six measurements were taken per line where a single measurement was performed on the middle part of three fully expanded leaves placed into the assimilation chamber. After measurements were completed, the leaf blade fragments were assessed for surface area, dry mass, and chlorophyll levels. In 2009, gas exchange data was acquired from three light treatments: full sun control, altered R:FR light, and canopy shade treatment, whereas in 2010 from four light treatments: full sun control, altered R:FR light, neutral shade, and canopy shade.

Model fitting

CO_2 exchange data were fitted by the equation of monomolecular function (Causton and Dale, 1990):

where, y is a photosynthetic rate at given x- PAR, *a* is an asymptotic maximum and here represents light saturated photosynthetic rate, *b* and *c* are slopes. From the equation dark respiration rate is calculated as $a(1-e^{b})$, light compensation point as b/c, and maximum quantum yield of photosynthesis (gradient at PAR=0) as ace^{b} (Fig.3.1). Nonlinear regression performed using PROC NONLIN of SAS (SAS Institute, Cary, NC) gave parameter estimates with standard errors. Overall, 12 data sets were fit for each line grown under full sun, altered R:FR light and canopy shade treatments, and 6 data sets for plants grown under neutral shade treatment. Analysis of variance was then performed on

acquired estimates using PROC MIXED of SAS (SAS Institute, Cary, NC) where analysis variable was weighted by its error value.

Quantitative determination of chlorophyll levels

Prior to chlorophyll analysis leaf samples were lyophilized and their dry mass was determined. The chlorophyll A and B content was then determined quantitatively in 5 ml N,N-Dimethylformamide extracts, and calculated from the absorption at the main bands (603 nm, 625 nm, 647 nm, 664 nm) (Moran, 1982).

Chlorophyll fluorescence

Chlorophyll fluorescence was measured using OS-30p Fluorometer (Opti-Sciences, Hudson, NH 03051). Maximum quantum yield of PSII was calculated as

$$Fv/Fm = [Fm-Fo]/Fm$$

where, Fo is a minimal fluorescence occurring when all antenna sites are assumed to be open and Fm is a maximal fluorescence (fluorescence under exposure to saturation flash) occurring when all antenna sites are assumed to be closed. For the measurement 5-8 leaves were taped using scotch tape and then dark incubated for 20 min using ‘dark adaptation’ clips (provided with fluorometer). In each experiment six measurements were taken for each line within each light treatment.

Stomatal density and stomatal size determination

Impressions of the adaxial and abaxial epidermis of middle part of fully expended leaves were made using the PVA glue films and light microscope slides. In each experiment, nine abaxial and nine adaxial impressions were taken per line. Two spots were randomly selected on each impression for the stomata count. Three stomata were selected on each leaf impression for size determination. Both stomatal number and size were determined under light microscope, using 40x and 100x magnification, respectively. The size of stomata was measured using MoticImages software. Following the assumption that stomatal aperture area is proportional to the guard cell length squared, the potential conductance index (PCI) was calculated as (Holland and Richardson, 2009):

$$PCI = (\text{guard cell length})^2 \times \text{stomatal density} \times 10^{-4}$$

Statistical analysis

Data were analyzed using PROC MIXED (SAS Institute, Cary, NC). Standard error of means (SEM) was used to calculate least significant differences (LSD) at the 0.05 probability level for means separation. Significant differences for the experiment with four light regimes were determined by analysis of variance (ANOVA) according to the split-split plot design for gas exchange experiment (light treatment as a main plot, genotype sub plot, and PAR level sub-sub plot), and split plot design for remaining experiments. Main effects of year, light and line as well as light by line interactions were tested.

RESULTS

Photosynthetic characterization

Since similar gas exchange results occurred, data for 2009 and 2010 were pooled for lines within light treatments.

Photosynthetic rates Overall *GA2ox* overexpression resulted in increased net photosynthetic rates in both modified plants. Line Ax6549 exhibit significantly (P=0.05) higher CO_2 uptake rates across all light treatments and at all PAR levels compared to Ax6548 and the NTC (Table 3.2; Fig. 3.2). For both canopy and neutral shade the differences in photosynthetic activity of Ax6549 and NTC were more pronounced at higher PAR levels. For reduced R:FR and full sun treatments, these differences were more consistent across all PAR levels. Photosynthetic rates of Ax6549 across all PAR levels averaged 39, 59, 89, and 54 % higher (P=0.05) than that of NTC grown in full sun, reduced R:FR, neutral shade, and canopy shade, respectively. Although Ax6548 exhibit increased photosynthetic rates this increase was not as remarkable equaling 6, 17, 40, and 9 % compared to NTC grown in full sun, reduced R:FR, neutral shade, and canopy shade, respectively. The 6 % increase in photosynthetic rate of Ax6548 grown in full sun was not significant.

The effect of light on plant's photosynthetic activity depended on both line and PAR level at which CO_2 uptake data was acquired. NTC grown under full sun treatment showed significantly higher (P=0.05) photosynthetic rates as compared to these grown in shade treatments when measured at 400 and 600 PAR level (Table 3.2; Fig. 3.3). No differences were found among shade treatments and lower PAR levels. In contrary, the

effect of light on CO_2 uptake rates of *GA2ox* overexpressing plants was apparent at all PAR levels. Overall, both Ax6549 and Ax6548 lines grown in neutral shade exhibit the highest photosynthetic rates, while no differences were found among the remaining light treatments.

Stomatal conductance The highest rates of photosynthesis in entry Ax6549 were concomitant with the highest (P=0.05) stomatal conductance (SC) as compared to Ax6548 and NTC (Table 3.4). No differences in SC were found between Ax6548 and NTC under any light treatment. Light did not have an effect on SC. A light by line interaction was not observed.

Dark respiration rates and light compensation points No differences in dark respiration rates (RD) and light compensation points (LCP) were found among plants grown under all light treatments (Table 3.3). Light treatments did not have an effect on these parameters and no light by line interaction was observed.

Maximum quantum yield of photosynthesis Analysis of maximum quantum yield of photosynthesis (MQYP) revealed that line Ax6549 had significantly higher MQYP under all light treatments compared to NTC plants. MQYP of Ax6549 was significantly (P=0.05) higher than that of Ax6548 under all light treatments except reduced R:FR light (Table 3.3). Despite the light treatment, no differences were detected between NTC plants and Ax6548. Light did not have an effect on MQYP and no light by line interaction was found.

Maximal photosynthetic rates Line Ax6549 had the highest maximal photosynthetic rates (MPR) among genotypes when grown under all light treatments (Table 3.3). Compared to NTC plants MPR of Ax6549 was 33, 69, 122, and 63 % higher

in full sun, reduced R:FR, neutral shade, and canopy shade, respectively. The MPR of Ax6548 was significantly (P=0.05) higher than that of NTC plants when grown under all shade treatments but not in full sun. The average increase in its MPR was 27, 56, and 17 % in plants grown under reduced R:FR light, neutral shade, and canopy shade treatments.

Although light had a significant effect on MPR, no light by line interaction was observed (Table 3.3). NTC plants grown in full sun treatment had significantly (P=0.05) higher MPR as compared to all shade treatments, no differences were noted among shade treatments. In Ax6549, a significant difference was detected only between plants from reduced R:FR environment and canopy shade treatment where MPR was significantly higher. Light did not have an effect on MPR of line Ax6548.

Stomatal density and size

Ax6549 had higher total stomata number (TSN) as compared to NTC when grown under all light treatments except neutral shade treatment (Table 3.4). TNC of Ax6549 was higher than that of Ax6548 when grown under full sun and reduced R:FR light treatment. No significant differences were found in the TSN of Ax6548 and NTC despite light treatment.

All plants responded to light treatments similarly (Table 3.4). In Ax6549 and NTC plants, TSN was decreased when plants were grown under canopy and neutral shade as compared to full sun and reduced R:FR light treatments. TSN of Ax6548 was the highest in plants grown under reduced R:FR light treatment. However, the difference was significant (P=0.05) only when compared to canopy shade treatment. No changes in ratio of stomatal number on adaxial and abaxial surfaces (SNR) were noted for Ax6548 and

Ax6549 despite light treatment, meaning that shade had the same effect on SN on both leaf surfaces (data not shown). SNR of NTC was significantly (P=0.05) lower in plants grown under full sun as compared to canopy and altered R:FR light treatments (data not shown).

Significant (P=0.05) differences in stomata length on both adaxial and abaxial leaf surfaces were found among the lines (Table 3.4). The adaxial surface stomata length (SL) of Ax6548 was lower than that of Ax6549 and NTC when grown under all light treatments. However, under canopy shade and reduced R:FR light treatments the differences were not significant (P=0.05) when compared to Ax6549 and NTC respectively. SL of Ax6549 differed from that of NTC only under neutral shade where it was significantly (P=0.05) lower. On the abaxial epidermis, SL of Ax6548 was lower as compared to Ax6549 and NTC when grown under canopy shade and full sun treatment. SL of Ax6549 was significantly (P=0.05) higher as compared to NTC for plants grown under neutral shade but lower for these grown under full sun treatment.

The effect of light on SL depended on the line (Table 3.4). In case of NTC on the adaxial side, greater SL was noted for plants grown under canopy and neutral shade as compared to reduced R:FR light and full sun treatments, whereas on the abaxial epidermis no differences in SL were found among the treatments. SL on the adaxial epidermis of Ax6548 was significantly (P=0.05) greater only when grown under canopy shade treatment. On the abaxial epidermis of Ax6548, SL was significantly higher for plants grown under neutral shade and the lowest under full sun treatment. For Ax6549 SL on adaxial epidermis of plants grown under reduced R:FR light was lower than that of plants grown in neutral shade. Abaxial SL of Ax6549 were higher in neutral shade as

compared to both reduced R:FR light and full sun treatments. Adaxial SL of plants grown in full sun was the lowest.

Since stomatal size and density both influence stomatal conductance, potential conductance indexes (PCI) were calculated (Table 3.4). PCI of Ax6549 was the highest, whereas that of Ax6548 the lowest despite light treatment. In Ax6549, PCI was increased by 15, 35, 14, and 16 % and in Ax6548 decreased by 30, 8, 10, and 9 % as compared to NTC grown under full sun, reduced R:FR, neutral, and canopy shade treatments, respectively.

Chlorophyll levels and chlorophyll fluorescence

Ax6548 maintained higher total chlorophyll levels when compared to Ax6549 and NTC when expressed on an area basis (Table 3.5). On average, total chlorophyll levels of Ax6548 were 49, 50, 65, and 31 % higher whereas these of Ax6549 were 6, 31, 32, and 12 % higher than those of NTC when grown under full sun, reduced R:FR light, neutral, and canopy shade, respectively. The 6 and 12 % increase in chlorophyll levels of Ax6549 grown in full sun and canopy shade was not significant (P=0.05). No significant (P=0.05) differences in chlorophyll A to chlorophyll B ratio (Ch A: Ch B) were found among the lines (Table 3.5).

Light had a significant effect on chlorophyll levels in transgenic plants but not in NTC (Table 3.5). Ax6548 had significantly (P=0.05) lower chlorophyll content when grown under canopy shade as compared to remaining light treatments. In Ax6549, the highest chlorophyll content was found in plants grown under reduced R:FR light treatment and was significantly (P=0.05) different when compared to that of plants form

canopy shade and full sun treatments. Light had a significant (P=0.05) effect on Ch A: Ch B of NTC and Ax6548 which was lower under canopy shade compared to remaining light treatments. Regardless of light treatments no significant (*P*=0.05) differences were found in Ch A:Ch B of Ax6549.

When expressed per mass unit, no differences in chlorophyll content were found among the lines grown under canopy shade (Table 3.5). Under neutral shade and reduced R:FR light treatments, Ax6549 had significantly lower chlorophyll content as compared to both Ax6548 and NTC. Under full sun treatment, chlorophyll content of Ax6549 was lower compared to Ax6548.

All plants maintained the lowest chlorophyll concentration per unit mass under full sun treatment (Table 3.5). Chlorophyll levels in full sun were significantly lower as compared to neutral shade in case of NTC and Ax6548 and canopy shade for Ax6549.

Plants differed significantly (*P*=0.05) in regards to the chlorophyll fluorescence (CF) (maximum quantum yield of photosystem II) (Table 3.5). Ax6548 had significantly higher CF compared to NTC when grown under all shade treatments. CF of line Ax6549 was higher than that of NTC under canopy and neutral shade. However, they did not differ under reduced R:FR light and full sun treatments. CF of Ax6548 was significantly higher than that of Ax6549 plants when grown under canopy shade and reduced R:FR light treatments.

Light by line interaction was observed in regards to CF (Table 3.5). In case of line Ax6549, CF of plants grown in canopy shade was significantly (*P*=0.05) higher as compared to reduced R:FR light and full sun treatments whereas that of plants grown under neutral shade was higher only as compared to reduced R:FR light treatment. Line

Ax6548 had the highest CF when grown under canopy shade and no differences were detected among remaining light treatments. In contrary CF of NTC was the highest under full sun treatment and the lowest under neutral shade and reduced R:FR light treatments.

Relationship of photosynthetic activity to chlorophyll content found that line Ax6549 had a higher chlorophyll use efficiency under all light treatments and PAR levels as compared to line Ax6548 and NTC (except PAR 50 and 100 of neutral shade treatment) (Table 3.2). Chlorophyll activity of Ax6548 was lower than that of NTC at all PAR levels for plants grown under full sun conditions and at lower (50-200) PAR levels for plants grown under reduced R:FR and neutral shade treatments. Chlorophyll activity of Ax6549 averaged 23, 34, 35, and 35 % higher while that of Ax6548 30, 22, 21, and 13 % lower compared to NTC grown in full sun, reduced R:FR, neutral shade, and canopy shade, respectively. The differences in chlorophyll activity between Ax6549 and NTC grown in canopy and neutral shade were increasing with increased PAR levels. Differences in unit chlorophyll activity of Ax6548 and control plant were diminishing together with increasing light intensity for plants grown in neutral shade and reduced R:FR environments but did not differ from each other across PAR levels for plants grown in full sun and neutral canopy shade.

Chlorophyll activity of lines differed among the light treatments. Ax6549 had lower (P=0.05) chlorophyll use efficiency in a reduced R:FR light environment when measured at PAR 400 and 600 (Table 3.2). When measured at 50 and 100 PAR, the CO_2 uptake rates per unit chlorophyll of Ax6548 were higher for plants grown in canopy and neutral shade compared to reduced R:FR light and full sun treatments while at higher PAR levels only as compared to reduced R:FR treatment. Ax6548 plants grown in full

sun and reduced R:FR treatments did not differ from each other with the respect to their chlorophyll use efficiency at any PAR level. For NTC significantly lower chlorophyll activity was noted in plants grown under reduced R:FR light as compared to neutral shade at PAR 50-200 and full sun at 400 and 600 PAR.

Specific leaf area

No differences in specific leaf area (SLA) were found among the plants grown under full sun treatment (Table 3.6). However line Ax6549 maintained significantly (P=0.05) lower SLA under all shade treatments, whereas Ax6548 under canopy and neutral shade as compared to NTC. SLA of transgenic plants did not differ significantly (P=0.05) from each other despite the light treatment.

Light had a significant (P=0.05) effect on SLA, and a light by line interaction was observed (Table 3.6). In case of NTC, plants grown under both canopy and neutral shade had the greatest whereas these from full sun treatment the lowest SLA. SLA of Ax6548 was the lowest when grown under full sun treatments and significantly different as compared to canopy and neutral shade plants. Significantly lower SLA was found in Ax6549 grown under full sun and reduced R:FR light as compared to canopy shade treatment.

Considering significant (P=0.05) variation in SLA among the plants gas exchange data were expressed per gram of leaf dry mass (Table 3.2). When expressed on a mass basis, photosynthetic activity of Ax6549 grown in full sun and canopy shade was on average 43 and 23 % higher (P=0.05) as compared to NTC. Ax6549 did not differ significantly from NTC when grown under reduced R:FR light and neutral shade

treatments. In full sun, increased photosynthetic activity of Ax6549 was observed at all PAR levels, while in canopy shade only at 400 and 600 PAR. No significant differences between CO_2 uptake rates of Ax6548 and NTC were observed under any light treatment and at any PAR level when considered per mass unit.

Similar responses, of photosynthetic activity of plants, to light treatments were observed despite line (Table 3.2). All examined plants grown under canopy and neutral shade treatment exhibit significantly (P=0.05) higher photosynthetic rates per gram of mass compared to these grown under reduced R:FR and full sun treatments. These differences were apparent at all PAR levels.

DISCUSSION

These studies showed that *PcGA2ox* overexpression resulted in increased single leaf photosynthetic rates in transgenic turfgrass plants as compared to NTC. Importantly, these differences were apparent at all PAR levels which suggest better use of low light by transgenic plants. This was partially in agreement with a study performed on tobacco plants overexpressing *AtGA2ox* which were characterized by higher CO_2 uptake rates however only at near saturated light intensities (Biemelt et al., 2004).

To this end, no studies have been performed to investigate the interactive effects of low light and gibberellin deficiency on photosynthetic capacity of plants. Here, higher single leaf photosynthetic rates of transgenic plants were maintained under shade treatments, and in the case of Ax6548, they became more apparent. Transgenic plants also differed in regard to their response to low light. While photosynthetic capacity (photosynthetic rates at saturating light intensity) of NTC leaves was lower under all

shade treatments, transgenic plants preserved their capacity for high photosynthetic rates. Ax6549 showed higher photosynthetic rates when grown under neutral shade treatment.

The effect of low irradiance on photosynthetic capacity was previously found to differ among and within turfgrass species. Lower light saturated photosynthetic rates as a response to shade were found in swards of 'Merion', 'Bartitia', and 'Limousine' Kentucky bluegrass, 'Pennlawn' red fescue, 'Tiflawn' bermudagrass, and leaves of tall fescue, and perennial ryegrass plants (Wilkinson et al., 1975; Winstead and Ward, 1974; Allard et al., 1991; Woledge, 1971; Woledge, 1977; Van Huylenbroeck and Van Bockstaele, 2001). In contrast, studies performed on St. Augustinegrass and 'Cindy' and 'Nevski' red fescue revealed increased rates of photosynthesis in these plants grown under reduced light intensity (Winstead and Ward, 1974, Van Huylenbroeck and Van Bockstaele, 2001). No differences in photosynthetic capacity of itchgrass were found between plants grown in high and low irradiance environment (Patterson, 1979). High CO_2 uptake rates of transgenic creeping bentgrass plants indicate a lack of adjustment of their photosynthetic apparatus to low light intensities and thus excessive allocation of resources into it as it was previously found in tall fescue (Allard et al., 1991).

Despite increased photosynthetic rates in transgenic plants no differences were found in leaf R_D rates and CP among lines under any light treatments except canopy shade where CP of entry Ax6549 was significantly lower than that of NTC. Both R_d rates and CP were not reduced under low light environments which was in contrary to experiments performed on tall fescue leaves where these parameters were slightly decreased when grown under shade conditions (Allard et al., 1991, Woledge, J., 1971). Ax6549 had higher MQYP (expressed in steeper initial slope of a curve) under all light

treatments, which suggests more efficient use of low light intensity by these plants. Significant increase in MQYP was previously found in gibberellin deficient tobacco plants (Biemelt, 2004). It was also shown by Bjorkman (1963, 1966) as a characteristic of shade adopted plants. However no differences in MQYP were found between species considered shade tolerant such as red fescue or shade intolerant such as Kentucky bluegrass (Wilkinson et al. 1975). In this study, shade treatments did not affect MQYP of NTC whereas both transformed plants had significantly higher MQYP when grown under neutral shade. Wilkinson et al. (1974) had previously found slight decrease whereas Allard et al. (1991) showed no change in MQYP in response to reduced light intensity. CF data showed that transgenic plants had higher efficiency of PSII photochemistry under all shade treatments in case of Ax6548, and neutral and canopy treatments for Ax6549. Neither *GA2ox* overexpressing line demonstrated higher PSII efficiency in full sun. Furthermore CF of Ax6548 was higher than that of Ax6549. This was in disagreement with photosynthetic data which revealed the highest photosynthetic rates in line Ax6549 and confirmed that chlorophyll fluorescence alone, performed outside laboratory setting, is not accurate measure of photosynthesis (Maxwell and Johnson, 2000).

Differences in photosynthetic characteristics among the plants were found to be accompanied by alterations in leaf anatomy and pigment levels caused by *GA2ox* overexpression. Previously, mesophyll volume and mesophyll cell size (Wilson and Cooper, 1967; Nobel et al., 1975, Allard et al. 1991), mesophyll density (Jellings and Leech, 1984, Allard et al., 1991), as well as stomata number (Bjorkman et al., 1972, Allard et al., 1991) were associated with maximum photosynthetic rates of leaves. On the

other hand, higher efficiency of low light absorption, a characteristic of shade ecotypes, was shown to be related with higher total chlorophyll levels per mass unit (Anderson et al., 1973), higher proportion of chlorophyll B (Goodchild et al, 1972), and larger grana stacks (Goodchild et al., 1972, Anderson et al., 1973).

Our data indicated lower specific leaf area (SLA) as one of the reasons for higher photosynthetic rates in transgenic plants. When photosynthetic data was recalculated and expressed on a mass basis, no differences were found between Ax6548 and NTC, despite light treatment and PAR levels. This indicates mesophyll volume as a main factor contributing to increased CO_2 uptake rates in these plants. A similar situation occurred in case of Ax6549 grown under reduced R:FR and neutral shade were its photosynthetic rates, expressed on mass basis, did not differ from those of Ax6658 and NTC plants. Under full sun and canopy shade treatments, CO_2 uptake rates per gram of tissue of Ax6549 were still higher than these of both Ax6548 and NTC. This was most likely caused by increased stomatal conductance in Ax6549 plants. Chlorophyll data showed that differences in net assimilation rates among lines were not due to chlorophyll levels. Further, Ax6548 had significantly higher levels of chlorophyll per mass unit compared to Ax6549 and NTC when grown under full sun conditions; however its CO_2 uptake rates were lower than those of Ax6549 and did not differ compared to NTC, indicating excessive assimilate allocation into chlorophyll biosynthesis in Ax6548.

Biemelt et al. (2004) demonstrated overexpression of *AtGA2ox* in tobacco plants resulted in increased CO_2 uptake rates concomitant with thicker spongy parenchyma layer, and additional but not complete, palisade layer. However, Huerta et al. (2008)

suggested more compact mesophyll tissue as a reason for increased photosynthetic rates in citrus plants overexpressing *CcGA20ox*.

CONCLUSIONS

Higher net photosynthetic rates in the leaves of transgenic plants imply higher net supply of photosynthate per unit leaf area in these plants. This could suggest better performance of both plants under shade conditions; however, that was not observed as only entry Ax6549 proved this supposition. Importantly transgenic plants differed in the magnitude of this increase with entry Ax6549 having significantly higher CO_2 uptake rates at all PAR levels. However, morphological analysis has to be performed to examine possible differences in assimilate allocation, and assess photosynthetic-respiratory balance of the plants.

Light treatment	**PPF** [mol photons m^{-2} d^{-1}] (% of full sun value)		**R:FR**
	2009	2010	2009/2010
Full sun	20.7 (100%)	28.5 (100 %)	1.28
Reduced R:FR	12.6 (60 %)	14.3 (50 %)	0.7
Reduced PPF (Neutral shade)	6.2 (30%)	8.5 (30 %)	1.28
Reduced PPF & R:FR (Canopy shade)	6.1 (30 %)	9.9 (35 %)	0.7

†Averaged daily PPF estimated from instantaneous measurements taken every 15 min in August-October 2009 and March- May 2010.

‡ Red to far red light ratio calculated from the wavelengths of 650-670 and 720-740 nm.

Table 3.1**:** Light conditions for August-October 2009 and March-May 2010.

	P_N								
	[µmol m^2]	[µmol g^{-1}]	[µmol m Ch^{-1}]	[µmol m^2]	[µmol g^{-1}]	[µmol mg Ch^{-1}]	[µmol m^2]	[µmol g^{-1}]	[µmol mg Ch^{-1}]
PAR	50			100			200		
	Full sun¶								
NTC	1.32†b‡*a*§	0.053b*c*	0.0042b*ab*	2.23b*a*	0.090b*c*	0.0072b*b*	3.90b*a*	0.157b*b*	0.0125b*ab*
Ax6548	1.31b*b*	0.055b*b*	0.0028c*b*	2.39b*b*	0.100ab*b*	0.0052c*b*	4.05b*b*	0.168b*b*	0.0087c*ab*
Ax6549	1.94a*ab*	0.079a*b*	0.0054a*a*	3.11a*c*	0.129a*b*	0.0089a*a*	5.56a*b*	0.222a*b*	0.0153a*a*
	Reduced R:FR#								
NTC	1.26b*a*	0.071ab*bc*	0.0038b*b*	2.21c*a*	0.124a*b*	0.0067b*b*	3.54c*a*	0.198a*b*	0.0108b*b*
Ax6548	1.26b*b*	0.057b*b*	0.0026c*b*	2.48b*b*	0.113a*b*	0.0050c*b*	4.05b*b*	0.184a*b*	0.0082c*b*
Ax6549	2.06a*ab*	0.079a*b*	0.0052a*a*	3.42a*ba*	0.133a*b*	0.0087a*a*	5.47a*b*	0.212a*b*	0.0140a*a*
	Neutral shade††								
NTC	1.31c*a*	0.116a*a*	0.0053ab*a*	2.30c*a*	0.208a*a*	0.0091a*a*	3.48c*a*	0.317a*a*	0.0135b*a*
Ax6548	1.75b*a*	0.105a*a*	0.0040b*a*	2.86b*a*	0.170a*a*	0.0064b*a*	4.72b*a*	0.277a*a*	0.0105c*ab*
Ax6549	2.12a*a*	0.109a*a*	0.0060a*a*	3.74a*a*	0.190a*a*	0.0105a*a*	6.29a*a*	0.317a*a*	0.0176a*a*
	Canopy shade‡‡								
NTC	1.27b*a*	0.090ab*b*	0.0043b*ab*	2.21c*a*	0.160ab*a*	0.0077b*ab*	3.73b*a*	0.272ab*a*	0.0130b*ab*
Ax6548	1.48b*ab*	0.085b*a*	0.0039b*a*	2.55b*ab*	0.147b*a*	0.0067b*a*	4.11b*b*	0.237b*a*	0.0108c*a*
Ax6549	1.80a*b*	0.104a*a*	0.0055a*a*	3.15a*bc*	0.183a*a*	0.0097a*a*	5.32a*b*	0.309a*a*	0.0163a*a*

†Within the column reported values are means of 12 measurements for full sun, altered R:FR, and canopy shade and 6 measurements for neutral shade.
‡Within columns means followed by the same letter are not significantly different at P=0.05.
§ Letters in italic indicate differences among the light treatments for one genotype.
¶ PPF=20.7 (2009) and 28.5 (2010) [mol photons m^{-2} d^{-1}] , R:FR=1.28.
# 60 % (2009) and 50 % (2010) of PPF value in full sun; R:FR=0.7
††30 % of PPF value in full sun; R:FR=1.28.
‡‡35 % (2009) and 30 % (2010) of PPF value in full sun; R:FR=0.7.

Table 3.2: Net photosynthetic rates (P_N) of nontransformed control (NTC), Ax6548, and Ax6549 grown under full sun, altered R:FR light, neutral shade, and canopy shade treatments, obtained at photosynthetic photon flux (PAR) of 50, 100, 200, 400, and 600 [µmols photons m^{-2} s^{-1}]**,** and expressed per area, mass and chlorophyll unit. **Continued on next page**

Table 3.2: **Continued**

	P_N					
	[µmol m^{-2}]	[µmol g^{-1}]	[µmol mg Ch^{-1}]	[µmol m^{-2}]	[µmol g^{-1}]	[µmol mg Ch^{-1}]
PAR	400			600		
	Full sun¶					
NTC	4.98†b‡*a*§	0.200b*b*	0.0160b*a*	5.27b*a*	0.211b*b*	0.0170b*a*
Ax6548	5.39b*ba*	0.224b*b*	0.0114c*ab*	5.68b*ab*	0.236b*b*	0.0119c*ab*
Ax6549	6.86a*b*	0.285a*b*	0.0197a*a*	7.34a*b*	0.303a*b*	0.0209a*ab*
	Altered R:FR#					
NTC	4.07c*b*	0.227a*b*	0.0124b*b*	4.24c*b*	0.237a*b*	0.0129b*b*
Ax6548	4.93b*b*	0.223a*b*	0.0100b*b*	5.16b*b*	0.233a*b*	0.0104b*b*
Ax6549	6.46a*b*	0.248a*b*	0.0167a*b*	6.96a*b*	0.271a*b*	0.0179a*b*
	Neutral shade††					
NTC	3.98c*b*	0.363a*a*	0.0154b*ab*	4.05c*b*	0.372a*a*	0.0156b*ab*
Ax6548	5.80b*a*	0.339a*a*	0.0129b*a*	5.97b*a*	0.350a*a*	0.0133b*a*
Ax6549	7.85a*a*	0.394a*a*	0.0219a*a*	8.34a*a*	0.420a*a*	0.0233a*a*
	Canopy shade ‡‡					
NTC	4.25c*b*	0.308b*a*	0.0148b*ab*	4.36c*b*	0.316b*a*	0.0152b*ab*
Ax6548	4.92b*b*	0.284b*a*	0.0129b*a*	5.14b*b*	0.296b*a*	0.0135b*a*
Ax6549	6.79a*b*	0.393a*a*	0.0207a*a*	7.22a*b*	0.418a*a*	0.0220a*a*

Parameter	R_D	MQYP	CP	Amax
	µmol CO_2 m^{-2} s^{-1}	µmol CO_2 $µmol^{-1}$ quanta	µmol quanta	µmol CO_2 m^{-2} s^{-1}
		Full sun¶		
NTC	0.45†a‡*a*§	0.040b*a*	11.2a*a*	5.95b*a*
Ax6548	0.46a*ab*	0.040b*b*	11.2a*ab*	6.30b*a*
Ax6549	0.48a*a*	0.051a*b*	9.1a*a*	7.92a*ab*
		Altered R:FR#		
NTC	0.56a*a*	0.043b*a*	11.4a*a*	4.10c*b*
Ax6548	0.72a*a*	0.049ab*a*	13.6a*a*	5.22b*a*
Ax6549	0.46a*a*	0.055a*ab*	7.9a*a*	6.95a*b*
		Neutral shade††		
NTC	0.49a*a*	0.045b*a*	10.7a*a*	3.96c*b*
Ax6548	0.38ab	0.051b*a*	6.5a*b*	6.18b*a*
Ax6549	0.53a*a*	0.063a*a*	8.0a*a*	8.82a*a*
		Canopy shade‡‡		
NTC	0.63a*a*	0.046b*a*	13.5a*a*	4.62c*b*
Ax6548	0.49a*ab*	0.046b*ab*	9.9ab*ab*	5.40b*a*
Ax6549	0.47a*a*	0.054a*ab*	8.9b*a*	7.55a*ab*

†Reported values are means of 12 estimates for full sun, altered R:FR, and canopy shade, and 6 estimates for neutral shadacquired by fitting monomolecular function to measured data.
‡Within columns means followed by the same letter are not significantly different at P=0.05.
§ Letters in italic indicate differences among the light treatments for one genotype.
¶ PPF=20.7 (2009) and 28.5 (2010) [mol photons m^{-2} d^{-1}] , R:FR=1.28.
# 60 % (2009) and 50 % (2010) of PPF value in full sun; R:FR=0.7
††30 % of PPF value in full sun; R:FR=1.28.
‡‡35 % (2009) and 30 % (2010) of PPF value in full sun; R:FR=0.7.

Table 3.3: Dark respiration rates (R_D), maximum quantum yield of photosynthesis (MPYP), light compensation points (CP), and maximal photosynthetic rates (A_{max}) of nontransformed control (NTC), Ax6548, and Ax6549 grown under full sun, altered R:FR light, neutral shade, and canopy shade.

Table 3.4: Stomatal conductance (SC), stomata number on adaxial (SN adaxial) and abaxial (SN abaxial) leaf surface, total stomata number (TSN), stomata length on adaxial (SL adaxial) and abaxial (SL abaxial) leaf surface, and potential conductance index (PCI) of nontransformed control (NTC), Ax6548, and Ax6549, grown in full sun, altered R:FR light, neutral shade, and canopy shade.

Table 3.4

Parameter	SC mmol m^{-2} s^{-1}	SN adaxial	SN abaxial	TSN no mm^{-2}	SL adaxial	SL abaxial µm	PCI
			Full sun§§				
NTC	0.094†b*a*	159.2¶ b*b*	104.3¶ a*a*	263.5¶ b*a*	25.0#a*b*	28.8#a*a*	18.8††
Ax6548	0.078b*a*	161.3b*a*	84.4a*a*	245.7b*ba*	23.3b*b*	22.6c*c*	13.1
Ax6549	0.154a*a*	221.4a*a*	95.2a*b*	316.3a*a*	26.3a*ab*	26.0b*c*	21.7
			Altered R:FR‡‡				
NTC	0.065b*b*	185.2a*a*	73.1b*b*	258.3b*a*	24.4ab*b*	27.9a*a*	16.7
Ax6548	0.086b*a*	181.3a*a*	81.6b*ba*	263.8b*a*	23.1b*b*	26.2a*b*	15.3
Ax6549	0.150a*a*	206.6a*a*	112.7a*a*	320.3a*a*	25.6a*b*	28.5a*b*	22.7
			Neutral shade¶¶				
NTC	0.061b*b*	142.3a*b*	71.2a*b*	213.1a*b*	28.9a*a*	27.9b*a*	17.4
Ax6548	0.087b*a*	161.3a*a*	73.3a*ba*	234.4a*ab*	24.2c*b*	29.2ba*a*	15.7
Ax6549	0.145a*a*	169.8a*b*	73.8a*c*	243.5a*b*	27.1b*a*	31.5a*a*	19.8
			Canopy shade##				
NTC	0.085b*ab*	144.0b*b*	57.7a*c*	201.7b*b*	28.0a*a*	29.5a*a*	16.3
Ax6548	0.087b*a*	155.9ab*a*	69.0a*b*	224.9ab*b*	25.8b*a*	25.6b*bc*	14.9
Ax6549	0.155a*a*	179.1a*ab*	67.3a*c*	249.3a*b*	26.9ab*ab*	29.8a*ab*	18.9

†Within the column reported values are means of 12 measurements for full sun, altered R:FR, and canopy shade and 6 measurements for neutral shade.

‡Within all columns means followed by the same letter are not significantly different at P=0.05.

§ Letters in italic indicate differences among the light treatments for one genotype.

¶ Within column reported values are means of 36 measurements for full sun, altered R:FR light, and canopy shade treatments, and 18 measurements for neural shade treatment.

Within the column reported values are means of 54 measurements for full sun, altered R:FR, and canopy shade treatment, and 27 measurements for neutral shade.

††Single value calculated as: PCI = (guard cell length)^2 x stomatal density x 10^{-4}**,** using average stomata size and number. Separate PCI was calculated for abaxial and adaxial leaf surfaced and summarized to represent total PCI.

‡‡PPF=20.7 (2009) and 28.5 (2010) [mol photons m^{-2} d^{-1}] , R:FR=1.28.

§§60 % (2009) and 50 % (2010) of PPF value in full sun; R:FR=0.7.

‡‡30 % of PPF value in full sun; R:FR=1.28.

¶¶35 % (2009) and 30 % (2010) of PPF value in full sun; R:FR=0.7.

Parameter	Ch A		Ch B		Ch T		Ch A: Ch B	Fv/Fmax
	mg m^{-2}	mg g^{-1}	mg m^{-2}	mg g^{-1}	mg m^{-2}	mg g^{-1}		
				Full sun¶				
NTC	244.5†b‡*a*§	9.6a*c*	81.6b*a*	3.2b*b*	327.2b*a*	13.0b*c*	3.1a*b*	0.787a*a*
Ax6548	368.8a*a*	14.9a*b*	118.3a*a*	4.8a*a*	487.4a*a*	19.7a*b*	3.2a*b*	0.784a*b*
Ax6549	260.7b*b*	10.9a*b*	86.2b*b*	3.6b*b*	347.4b*b*	14.8b*b*	3.0a*a*	0.780a*bc*
				Reduced R:FR#				
NTC	249.5c*a*	11.6a*c*	81.2c*a*	4.6ab*a*	330.2ca	18.4ab	3.2a*b*	0.769b*c*
Ax6548	374.6a*a*	14.0a*b*	120.9a*a*	5.5a*a*	495.2a*a*	22.5a*ab*	3.2a*b*	0.787a*b*
Ax6549	328.5b*a*	9.9a*b*	104.7b*a*	4.0b*ab*	433.8b*a*	16.1b*ab*	3.2a*a*	0.773b*c*
				Reduced PPF††				
NTC	215.4c*a*	21.5a*a*	66.6b*a*	5.7a*a*	281.6c*a*	26.4a*a*	3.7a*a*	0.765b*c*
Ax6548	368.2a*a*	22.3a*a*	96.5a*b*	5.5a*a*	465.3a*a*	27.1a*a*	4.0a*a*	0.779a*b*
Ax6549	286.7b*ab*	14.8a*ab*	85.4a*b*	4.2b*ab*	372.3b*ab*	18.2b*ab*	3.6a*a*	0.785a*ab*
				Reduced R:FR&PPF‡‡				
NTC	222.0b*a*	16.1a*b*	75.3b*a*	5.3a*a*	296.7b*a*	21.4a*ab*	3.1a*b*	0.777c*b*
Ax6548	289.9a*b*	16.9a*b*	97.1a*b*	5.5a*a*	387.6a*b*	22.4a*ab*	3.2a*b*	0.798a*a*
Ax6549	251.3ba*b*	14.9a*a*	80.1b*b*	4.8a*a*	331.5b*b*	19.8a*a*	3.3a*a*	0.789b*a*

† Within the column reported values are means of 12 measurements for full sun, altered R:FR, and canopy shade, and 6 measurements for neutral shade.
‡Within columns means followed by the same letter are not significantly different at P=0.05.
§ Letters in italic indicate differences among the light treatments for one genotype.
¶ PPF=20.7 (2009) and 28.5 (2010) [mol photons m^{-2} d^{-1}] , R:FR=1.28.
# 60 % (2009) and 50 % (2010) of PPF value in full sun; R:FR=0.7
††30 % of PPF value in full sun; R:FR=1.28.
‡‡35 % (2009) and 30 % (2010) of PPF value in full sun; R:FR=0.7.

Table 3.5: Chlorophyll A (Ch A), chlorophyll B (Ch B), and total chlorophyll (Ch T) content expressed per area and mass unit, chlorophyll A to chlorophyll B ratio (Ch A : Ch B), and chlorophyll fluorescence (Fv/Fmax) of nontransformed control (NTC), Ax6548, and Ax6549, grown under full sun, reduced R:FR light, neutral shade, and canopy shade treatments.

Parameter	SLA
	[m^2 g^{-1}]
	Full sun¶
NTC	0.041†a*c*§
Ax6548	0.042a‡*b*
Ax6549	0.042a*b*
	Reduced R:FR#
NTC	0.056a*b*
Ax6548	0.046ab*ab*
Ax6549	0.039b*b*
	Neutral shade††
NTC	0.090a*a*
Ax6548	0.059b*a*
Ax6549	0.050b*ab*
	Canopy shade‡‡
NTC	0.074a*a*
Ax6548	0.058b*a*
Ax6549	0.058b*a*

† Within the column reported values are means of 12 measurements for full sun, altered R:FR, and canopy shade, and 6 measurements for neutral shade.
‡Within columns means followed by the same letter are not significantly different at *P*=0.05.
§ Letters in italic indicate differences among the light treatments for one genotype.
¶ PPF=20.7 (2009) and 28.5 (2010) [mol photons m^{-2} d^{-1}] , R:FR=1.28.
# 60 % (2009) and 50 % (2010) of PPF value in full sun; R:FR=0.7
††30 % of PPF value in full sun; R:FR=1.28.
‡‡35 % (2009) and 30 % (2010) of PPF value in full sun; R:FR=0.7.

Table 3.6 Specific leaf area (SLA) of nontransformed control (NTC), Ax6548, and Ax6549 grown under full sun, reduced R:FR light, neutral shade, and canopy shade.

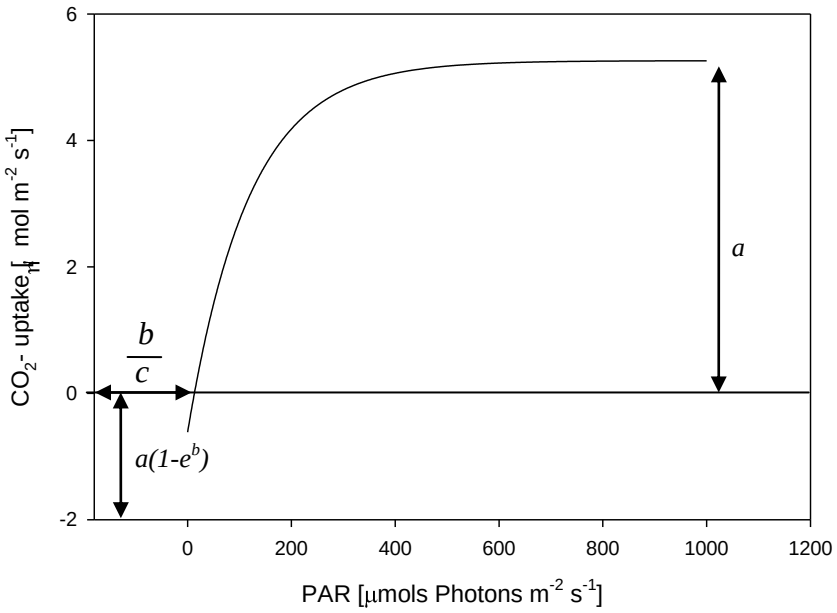

Fig. 3.1: Features of monomolecular function. Asymptote *a* represents light saturated photosynthetic rate, *x* axis intercept (*b/c*) represents light compensation point, and *y* axis intercept ($a(1-e^b)$) represents dark respiration rate.

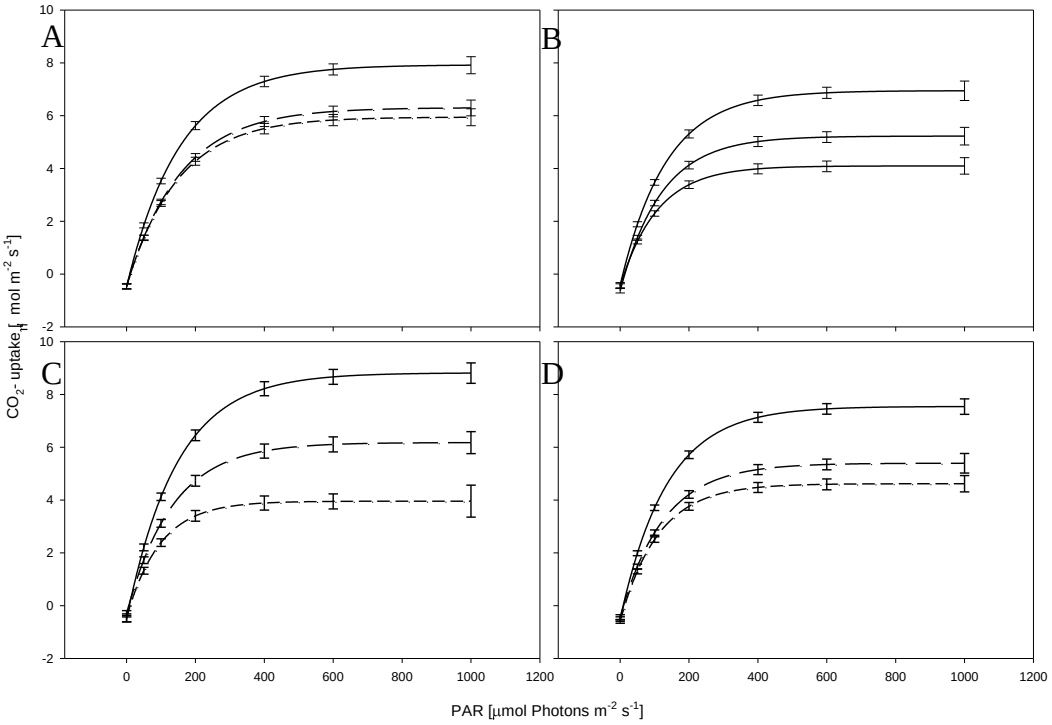

Fig. 3.2: CO_2 light response curves of nontransformed control (short dash line), Ax6548 (long dash line), and Ax6549 (solid line) grown under full sun (A), reduced R:FR (B), neutral shade (C), and canopy shade (D) treatments. Each curve represents the average of 12 data sets for full sun, reduced R:FR, and canopy shade, and 6 data sets for neutral shade. Error bars designate standard error.

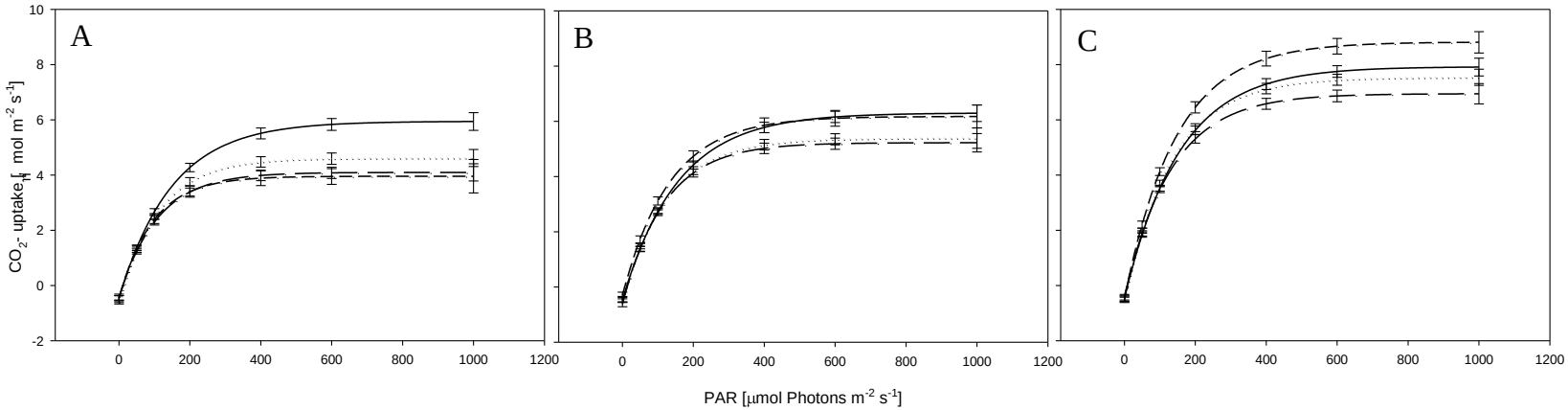

Fig. 3.3: CO_2 light response curves of nontransformed control (A), Ax6548 (B), and Ax6549 (C) grown under full sun (solid line), altered R:FR light (long dash line), neutral shade (short dash line), and canopy shade (dotted line) treatments. Each curve represents the average of 12 data sets for full sun, reduced R:FR, and canopy shade, and 6 data sets for neutral shade. Error bars designate standard error.

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CHAPTER 4

THE EFFECT OF *PCGA2OX* OVEREXPESSION ON THE BIOMASS PARTITIONING IN CREEPING BENTGRASS (*AGROSTIS STOLONIFERA* L.) GROWN IN FULL SUN AND REDUCED LIGHT

ABSTRACT

Creeping bentgrass (*Agrostis stolonifera* L.) is a species commonly used in high maintenance turf areas, where it is often exposed to shade. When grown under reduced irradiance, turfgrass plants exhibit shade avoidance response that ultimately leads to the loss of turfgrass coverage. Adaptive responses to light stimuli are mediated, in part, by phytohormones gibberellins (GA). Biotechnological manipulation of GA levels has been successfully utilized in modifying plant stature. The main objective of this study was to determine the effect of genetically induced dwarfism, by *GA2ox* overexpression, on creeping bentgrass assimilate partitioning in light limited environments. Morphological and growth measurements were taken under controlled environment conditions. Two transgenic lines, Ax6548 and Ax6549, transformed with *PcGA2ox* and *CP4 EPSPS* genes, and nontransformed control plants were subjected to high and low irradiance environments. Biomass accumulation was reduced in both transgenic plants. Ax6549 plants were characterized by lower growth efficiency and leaf biomass production

efficiency which was linked to a potential increase in self shading in these plants. Changes in assimilate partitioning were observed in transgenic plants with Ax6549 partitioned more assimilates into roots and Ax6548 into leaves. Despite examined line, shade treatment caused reduction in biomass production, and increased both leaf area ratio and specific leaf area. Under shaded condition all plants invested more resources into leaves at the expense of roots. Both modified plants partitioned more assimilates into leaves compared to NC plants. Morphological data collected did not clarify the improved performance of Ax6549 under shaded conditions

INTRODUCTION

Under low light conditions, sun adapted species undergo a series of morphological and physiological changes. To maximize light interception, shaded plants partition more biomass into vertical growth (e.g., elongated internodes and petioles) which is often acquired at the expense of resource allocation to lateral branching and leaf and root biomass (Henry and Aarssen, 1997; Hebert et al., 2001; Huber and Wiggerman, 1997). In addition to the effects of shade on plant architecture, leaf anatomy and morphology can also be affected leading to the production of larger but thinner leaves (Corre, 1983; Allard et al., 1991; Wherley et al., 2005).

Turfgrasses require four to six hours of full sun per day (Fry and Huang, 2004). When grown under low light, turfgrasses and other sun-adapted plants exhibit shade avoidance response. In shade studies performed on bermudagrass, low irradiance caused elongation of stolons and reduced branching (Dong and de Kroon, 1994). Wherley et al.

(2005) reported that tall fescue plants grown in full sun produced five times more root biomass, and 1.5 and 3.5 more tillers than those grown under neutral shade and deciduous shade, respectively. Detailed growth analysis showed that a common response of C3 and C4 grasses to shade included increased leaf area ratio (LAR), increased shoot-to-root ratio, and decreased specific leaf weight (SLW) and reduced total plant dry mass (Allard et al. 1991; Kephart, 1992). Allard et al. (1991) reported that tall fescue grown in shade developed leaves that were on average, 54 to 65 % longer, consisted of 56 to 77 % more leaf area, but were 12 % thinner.

Although aforementioned adaptive changes to low light may be beneficial in natural ecosystems, they are ineffective and undesirable traits in high maintenance turfgrass settings. In golf and sport turf situations under limited light, frequent mowing at short heights will decrease photosynthetic capacity and ultimately decrease turf quality.

Adaptive changes in plants due to alterations in light quality, quantity and duration are mediated, in part, by the phytohormones, gibberellins (GA) (Hadden and Kamiya, 1997; Sponsel and Hadden, 2004). Manipulation of GA levels by overexpression of genes involved in their catabolism has been successfully utilized to modify plant stature. Overexpression of *GA2ox* resulted in dwarf phenotype in many plants including rice (Sakamoto et al., 2001.), tobacco (Schomburg et al., 2003; Biemelt et al., 2004), poplar trees (Busov et al., 2003), Arabidopsis (Radi et al., 2006), and bahiagrass (Agharkar et al., 2007). Transgenic rice plants overexpressing *OsGA2ox1* gene were characterized by shorter but wider leaf blades (Sakamoto et al, 2001). Overexpression of *AtGA2ox1* (Agharkar et al., 2004) in bahiagrass resulted in plants with increased number of tillers and

reduced leaf and stem length. However, total biomass as well as shoot or root biomasses were not affected.

Dwarf creeping bentgrass plants containing runner bean (*Phaseolus coccineus*) *GA2- oxidase* gene (*PcGA2ox*) have been created (Yan, 2006). Previous studies performed on these plants showed line Ax6549 to have superior quality compared to nontransformed control under reduced light conditions. In addition Ax6549 was characterized by significantly higher single leaf photosynthetic rates compared to control plant. The objective of this study was to investigate the effect of dwarfism on creeping bentgrass assimilate partitioning under high and low irradiance environments.

MATERIALS AND METHODS

Plant material and growth conditions

Two transgenic creeping bentgrass *(Agrostis stolonifera* L.*)* lines, Ax6548 and Ax6549, transformed with runner bean *(Phaseolus coccineus*) *GA2- oxidase* (*PcGa2ox*) and *CP4 EPSPS* genes, and nontransformed control (NTC) were included in the study. Plants were propagated in tubes (6 x 25 cm) filled with 100 % sand, using single rooted tillers of uniform size. For propagation, tillers and roots were trimmed to 2 cm. For the first study plants were allowed to establish in the greenhouse for 9 wk (30 March – 1 June) while for the second study for 7 wk (12 May – 1 July 2010). After establishment, plants were transferred to controlled environment chambers. During the establishment plants were maintained at 2 cm height. The plants were fertilized at the rate of 0.2 kg of N per 92.9 m^2, using 100 ppm solution of 20N-10P-20K 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-imidazolidine-carboxyamide) 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 insecticides were performed when needed. Irrigation was applied to prevent wilt.

Treatments and experimental design

The studies were conducted at The Ohio State University, Department of Horticulture and Crop Science, Columbus, Ohio. Plants were randomly assigned to two light treatments: high irradiance (HI) treatment with 20 h photoperiod of 500 µmol photons m^{-2} s^{-1}, and reduced irradiance (RI) treatment with 20 h photoperiod of 175 µmol photons m^{-2} s^{-1} provided by fluorescent lamps. Both chambers were maintained at 20/15^{o} C day (20 h) and night (4 h) temperature.

The experiment was arranged as split-plot with 8 replicates where irradiance treatments served as a main plots and creeping bentgrass lines as subplots. Samples were taken on day 1 of the experiment and then every 10 days for next 30 days. On each sampling date, 8 plants per genotype were harvested, and dissected into leaf blades, stems (leaf sheets and stolons), and roots. Ten 2 cm long middle leaf sections were sampled from each plant for the area and mass determination. The leaf area:mass ratio was then used to estimate total leaf area of the plant. Samples were dried in a forced air oven at 60^{o} C for 72 hours. Subsequently, the root, leaf and stem mass were determined.

Quantitative growth analysis

Plant growth analysis was performed using formula from Radford (1967):

- Crop growth rate (CGR) [mg d^{-1}] ($W2 - W1$)/($T2 - T1$)
- Relative growth rate (RGR) [mg g^{-1} d^{-1}] (log$eW2$ − log$eW1$)/($T2 − T1$)
- Net assimilation rate (NAR) [g m^{-2} d^{-1}] ($W2 − W1$)/(T 2 − T 1)[(log LA 2 − log LA 1)/(LA 2 − LA 1)]
- Leaf area ratio (LAR) [m^2 g^{-1}] [(LA 1/$W1$) + (LA 2/$W2$)]/2
- Specific leaf area (SLA) [m^2 g^{-1}] [(LA 1/LW1) + (LA 2/LW2)]/2
- Leaf weight ratio (LWR) [g g^{-1}] [(LW1/$W1$) + (LW2/$W2$)]/2
- Stem weight ratio (SWR) [g g^{-1}] [(SW1/$W1$) + (SW2/$W2$)]/2

Where:

W - total dry weight of the plant, T - time (d), LA - leaf area, LW - dry weight of leaf blade, SW - dry weight of stem, RW - dry weight of root.

Statistical analysis

Effects of light and genotype were analyzed by analysis of variance according to PROC MIXED procedure of the Statistical Analysis System (PC version 9.1, the SAS Institute, Cary, NC). Standard error of means (SEM) was used to calculate least significant differences (LSD) at the 0.05 probability level for means separation.

RESULTS

Data reported here is from July study. Data from June study is found in appendix.

Crop growth rate

Analysis of variance indicated that light, line, and time of sampling had significant effect on crop growth rate (CGR). Moreover an interaction between line and sampling time was detected. Under HI during first ten days of the study, Ax6549 maintained 190 and 334 % higher (P=0.05) CGR as compared to NTC and Ax6548 plants, respectively (Table 4.1, Fig. 4.3). However, over the next 20 days, both NTC and Ax6548 grew at a faster pace, and by the end of the study, CGR of Ax6549 was 32 % lower (P=0.05) compared to NTC plants and did not differ from that of Ax6548. A similar trend was observed under RI however, differences among genotypes were less pronounced (Table 4.1, Fig. 4.4).

All plants maintained higher CGR under HI as compared to RI environment (Table 4.1). NTC plants had significantly (P=0.05) lower CGR under RI for the second (49 %) and third (26 %) sampling periods. CGR of Ax6548 was significantly (P=0.05) lower under RI during second sampling period (44 %), while that of Ax6549 was lower during first (71 %) and second (37 %) sampling period. The differences in CGR between light treatments were least pronounced for the last 10 days of the study.

Relative growth rate

To account for the initial plant mass, relative growth rates (RGR) were obtained by CGR data transformation with natural logarithm. Statistical analysis of RGR showed

significant effects of light, line, and sampling time, as well as line by sampling time interaction. Under HI conditions, no differences in RGR were found among lines for the first 10 days of the study (Table 4.1). During the second sampling period, RGR of Ax6549 was 52 and 47 % lower (P=0.05) than that of NTC and Ax6548 plants, respectively. These differences were due to significant (P=0.05) decrease in RGR for Ax6549 and increase for NTC and Ax6548 plants. RGR of Ax6549 calculated for last 10 days of the study was 39 % (P=0.05) lower compared to Ax6548 and did not differ from that of NTC plants. No differences were found between NTC and Ax6548 plants in regards to RGR at any sampling period. Under HI all plants had the lowest RGR during last 10 days of the study. Under RI treatment RGR of Ax6549 was 35 and 32 % lower (P=0.05) during first and last sampling period of the experiment as compared to Ax6548, and 31 % lower during second sampling period as compared to NTC plants (Table 4.1).

During first sampling period all plants exhibit lower RGR under RI compared to HI (Table 4.1). The reduction in RGR equaled 39, 25, and 57 % for NTC, Ax6548, and Ax6549, respectively. Lower RGR of NTC and Ax6548 under RI were maintained during second sampling period however they were less pronounced. No differences in RGR values were found between HI and RI for last 10 days of the study.

Net assimilation rate

Net assimilation rates (NAR) were affected by line, light, and sampling time. The effect of line depended on the sampling time. Under HI conditions during first sampling period, dry matter production per leaf area for Ax6549 was 66 and 27 % higher (P=0.05)

compared to NTC and Ax6548 plants, respectively (Table 4.2). During the second and third sampling periods, NAR was the highest (P=0.05) for NTC plants and the lowest (P=0.05) for Ax6549. Dry matter production efficiency of leaves of Ax6548 grown in HI was more consistent across the study.

Under RI, significant differences were detected among the plants only for first sampling period where NAR obtained for Ax6549 was lower than that of Ax6548. RI had a negative effect on dry matter production efficiency despite examined genotype and sampling period. Similar results were observed when NAR was expressed per unit leaf mass (Table 4.2.).

Biomass partitioning- root, shoot, and leaf weight ratio

Analysis of variance indicated that both root weight ratio (RWR) and leaf weight ratio (LWR) were affected by line, light, and time of sampling. Also light by line and line by time interaction occurred. In regards to stem weight ratio (SWR), the effect of line and time as well as line by time interaction were observed. Light did not have an effect on SWR.

On day one of the study, both NTC and Ax6548 partitioned significantly (P=0.05) more (13 and 14 %, respectively) resources into roots (RWR) as compared to Ax6549 (Table 4.3; Fig. 4.1). On the other hand, Ax6549 invested 10 and 14 % more (P=0.05) dry matter into stems (SWR) compared to NTC and Ax6548, respectively. Both transgenic plants invested 3 % more (P=0.05) biomass into leaves (LWR) than NTC plants. After subjecting plants to HI treatment, both NTC and Ax6548 partitioned more

assimilate into stems and leaves at the expense of roots. During the first 10 days of the study, NTC and Ax6548 invested 25 and 24 % less biomass into roots, respectively. At that time, Ax6548 partitioned 10 % more assimilate into steams and 14 % more into leaves while NTC plants 8% more into stems and 17 % more into leaves. In contrast marginal changes in assimilate partitioning of Ax6549 grown under HI were observed over the duration of the study. These different responses resulted in significantly (P=0.05) higher RWR in Ax6549 on 2nd, 3rd, and 4th sampling date as compared to NTC, but only on 2nd and 3rd sampling date as compared to Ax6548. In regards to SWR, no differences among the plants were observed on the second sampling date. However, NTC plants maintained higher (P=0.05) SWR on 3rd and 4th sampling date as compared to both *GA2ox* lines. Ax6548 partitioned significantly (P=0.05) more assimilates into leaves than Ax6549 on 2nd (12 %), 3rd (10 %), and 4th (4 %) sampling date and NTC plants on 3rd (8 %) and 4th (4 %) sampling date.

Plants differed in their initial response to RI (Table 4.3; Fig. 4.2). During the first 10 day period, similar changes in assimilate partitioning in NTC and Ax6548 occurred under RI compared to HI treatment (Table 4.3). However, different response to RI was observed in Ax6549 plants. At that time NTC and Ax6548 plants allocated 29 and 23 % less biomass into roots and redirected their resources into stems (9 and 8 % for NTC and Ax6548, respectively) and leaves (20 and 15 % of NC and Ax6548, respectively). Ax6549 invested 14 % more resources into leaves at the expense of roots. As a result on 2nd sampling date RWR of Ax6548 was higher while its SWR lower as compared to NTC and Ax6549. No differences in LWR were observed among lines at that time. Over next

10 days, RWR of NTC plants and Ax6548 continued to decline at a greater pace compared to Ax6549 and at 3rd sampling date RWR of NTC plants was significantly (*P*=0.05) lower than that of Ax6549. On the 3rd sampling date NTC plants invested 9 and 10 % more (*P*=0.05) resources into stems compared to Ax6548 and Ax6549, respectively. LWR of NTC plants measured on 3rd sampling date was significantly (*P*=0.05) lower compared to both transgenic plants. On the last sampling date no differences were found in RWR among the genotypes grown under RI. SWR of NTC plants was higher (*P*=0.05) than that of Ax6549. LWR of both transgenic plants was higher (P=0.05) than that of NTC plants.

Overall RI treatment had negative effect on RWR of all plants (Table 4.3). However, the decline in relative root mass occurred on different dates. In case of Ax6549 lower RWR was observed 10 days after subjecting plants to RI. For Ax6548 this response was delayed by 20 days. RWR of NTC plants grown under RI was lower only on third sampling date. Shade treatment did not have an effect on SWR of genotypes except 4th sampling date when SWR of Ax6548 was significantly lower (*P*=0.05) compared to HI. All plants partitioned more (*P*=0.05) assimilates into leaves when grown under reduced light conditions. However, in case of Ax6549, the increase in LWR was noted 10 days after subjecting to RI while for NTC and Ax6548 after 20 days.

Leaf area ratio

Leaf area ratio (LAR) was affected by both light treatments and sampling time. Light by time, light by line as well as line by time interactions were detected. On day 1 of

the study Ax6549 had 25 % higher LAR as compared to Ax6548 (Table 4.4). Under HI treatment LAR of both NTC and Ax6548 increased substantially (67 and 50 %, respectively) during the first 10 days. The initial spike in LAR observed for NTC on the second sampling day was followed by a decrease (47 %) while both transgenic plants maintained their LAR at the same level. On 3rd sampling date Ax6548 had 50 and 33 % higher (P=0.05) LAR as compared to NTC and Ax6549, respectively. However, over next 10 days LAR of Ax6548 dropped and thus by the end of the study no differences in this parameter were detected among the lines.

During first 10 days under RI treatment, LAR of NTC, Ax6548, and Ax6549 increased by 133, 125, and 90 %, respectively. On 2nd sampling date LAR of NTC plants was 16 and 11 % higher (P=0.05) than that of Ax6548 and Ax649, respectively (Table 4.4). However due to slight changes in LAR of all plants that occurred over next 10 days, no differences were detected among the plants on 3rd sampling date. Although during last 10 days of the study LAR of all plants declined, the greatest decrease was noted for NTC plants. At the end of the study, LAR of NTC was 26 % lower than that of transgenic plants.

Compared to HI, RI caused significant increase in LAR of all three lines (Table 4.4). Substantial increase in LAR was noted during first 10 days of the study. As compared to HI treatment on 2nd sampling date LAR of NTC, Ax6548, and Ax6549 grown in RI was 40, 50, and 111 % higher (P=0.05), respectively. Over next 10 days the differences between light treatments became more pronounced. At that time LAR of NTC, Ax6548, and Ax6549 grown under RI was 150, 75, and 133 % higher (P=0.05)

than that of HI plants, respectively. On the last sampling date LAR of NTC, Ax6548, and Ax6549 grown under RI was 100, 111, and 137 % higher as compared to corresponding values from HI treatment.

Specific leaf area

Analysis of variance indicated that specific leaf area (SLA) was affected by light and line. Effect of both light and line depended on sampling time. On day 1 of the study SLA of Ax6548 was 24 and 19 % lower (P=0.05) as compared to NTC and Ax6549, respectively (Table 4.4). After subjecting to HI over first 10 days of the study, SLA of all genotypes decreased. At the 2nd sampling date, SLA of Ax6548 was 20 % lower (P=0.05) than that of NTC plants. During next 10 days SLA of NTC plants continued to decrease and at 3rd and 4th sampling date no differences were detected among all lines.

Under RI treatment during first 10 days of the study SLA of all plants increased significantly (P=0.05) (Table 4.4). However, plants differed in the magnitude of the response with SLA of NTC, Ax6548, and AX6549 increased by 12, 34, and 15 %, respectively. Thus despite initial differences in SLA, on 2nd sampling date no differences were found among lines. On 3rd sampling date SLA of NTC plants was significantly higher compared to Ax6548. No differences were detected on 4th sampling date among lines.

At all sampling dates, SLA of all lines grown under RI was higher compared to HI treatment (Table 4.4). On second sampling date SLA of NTC, Ax6548, and Ax6549 grown under RI was 37, 55, and 46 % higher as compared to corresponding values from

HI treatment. Over next 10 days this difference further increased for NTC plants and on the third sampling date equaled 81 %. No changes were noted for both transgenic lines at that time. By the end of the study SLA of NTC, Ax6548, and Ax6549 grown under RI was 59, 56, and 51 % compared to HI treatment.

DISCUSSION

PcGA2ox overexpression resulted in decreased crop growth rates in transgenic creeping bentgrass plants compared to NTC plants. This was in agreement with studies performed by Biemelt et al. (2004) where tobacco plants overexpressing *AtGA2ox* gene were characterized by lower biomass production compared to control plants. In contrast no changes in total biomass were found in bahiagrass transformed with *AtGA2ox1* (Agharkar et al., 2007). Growth efficiency (RGR) data indicated absolute mass of the plants as one of the reasons for differences in growth rates. Leaf productivity (NAR) may be another determinant contributing to these variations. Although previous photosynthetic characterization of these plants showed significantly higher single leaf photosynthetic rates in Ax6549, in this study it occurred only for first sampling period. Leaf productivity, as derived from morphological analysis, was previously correlated with leaf blade width (Patton et al., 2007). Patton et al. (2007) concluded that higher dry matter production by leaves of 'Diamond' and 'Zorro' zoysiagrass as compared to 'Meyer' and 'El Toro' was due to narrower leaves and thus less self shading in. Ax6549 had the widest leaves and Ax6548 the most narrow leaf blades among the lines evaluated (Table 4.4). Previous studies performed by Yan (2006) showed that both lines, Ax6548

and Ax6549, were characterized by more horizontal leaf orientation. These two traits may act in concert to increase self shading of the leaves of Ax6549, particularly in matured, dense canopy.

In addition to differences in growth rates, *GA2ox* overexpression altered assimilate partitioning in transgenic plants. Although biomass partitioning varied over the duration of the study overall, both transgenic plants partitioned fewer resources into stems and more into roots, in case of Ax6549, and leaves for Ax6548. No changes in total root or shoot biomass were observed in bahiagrass transformed with *AtGA2ox* (Agharkar et al., 2007).

Shade caused typical changes in growth characteristics of all plants. As expected, overall biomass production (CGR) was lower under reduced light conditions. A significant increase in relative leaf biomass with concurrent decrease in relative root biomass was observed for all plants. This is a typical response and was previously noted for shade grown itchgrass (Patterson, 1979). Two parameters extensively studied in relation to shade are LAR and SLA. In agreement with our finding increase in LAR and SLA as a response to reduced light has been shown for a number of species including tall fescue and itchgrass (Patterson, 1979; Allard et al., 1991; Kephart and Buxton, 1993).

No studies were previously performed to investigate the effect of combined reduced irradiance and genetically induced dwarfism on the growth characteristics of plants. Here we demonstrated that although shade caused similar alterations in all plants, differences among genotypes were apparent. Overall transgenic plants invested more resources into leaves. Further plants differed in regards to the onset of changes. Shade

related alterations occurred the fastest in Ax6549 and were delayed by 10 days for NTC and Ax6548 plants.

CONCLUSIONS

Growth and morphological analysis did not provide clear cut results to elucidate potential factors underlying improved quality of Ax6549 under reduced light conditions. We previously concluded that higher net supply of assimilates from unit leaf area may assure better performance of Ax6549. Leaf productivity data did not confirm this finding. Although at first glance results from both studies seem contradictory it is important to note that single leaf photosynthetic measurements do not account for the canopy interactions. This is an important factor taken differences in leaf morphology and orientation and thus potential self shading of plants at higher cutting heights. Whole canopy photosynthetic measurements performed at different cutting heights are needed to further explain data discrepancy. Also altering shade intensities may help in proving more distinct growth and morphological data.

Line	**CGR** [mg d^{-1}]			**RGR** [mg $g^{-1}d^{-1}$]		
	1-10	**10-20**	**20-30**	**1-10**	**10-20**	**20-30**
	High irradiance†					
NTC	38.2b§*a*¶	231.2a*a*	349.8a*a*	104.7a*a*	150.8a*a*	71.6ab*a*
Ax6548	25.5b*a*	116.8c*a*	228.7b*a*	111.3a*a*	135.9a*a*	83.0a*a*
Ax6549	110.7a*a*	173.9b*a*	236.5b*a*	127.4a*a*	72.4b*a*	50.9b*a*
	Reduced irradiance‡					
NTC	20.1a*a*	116.9a*b*	260.5a*b*	63.9ab*b*	124.4a*b*	85.0ab*a*
Ax6548	16.9a*a*	65.9b*b*	189.4b*a*	83.6a*b*	103.8ab*b*	99.9a*a*
Ax6549	32.0a*b*	108.7ab*b*	197.1b*a*	54.3b*b*	85.6b*a*	68.0b*a*

† 20 h photoperiod of 500 µmol photons m^{-2} s^{-1}

‡20 h photoperiod of 175 µmol photons m^{-2} s^{-1}

§ Within columns means followed by the same letter are not significantly different at P=0.05.

¶ Letters in italic indicate differences between the light treatments for one genotype.

Table 4.1: Crop growth rate (CGR) and relative growth rate (RGR) of nontransformed control (NTC), Ax6548, and Ax6549 (n=8) calculated for day 1 to 10, 10 to 20, and 20 to 30 periods of July study, for plants grown under high irradiance and reduced irradiance treatments.

Line	**NAR** [g m^{-2} d^{-1}]			**NAR** [mg g^{-1} d^{-1}]		
	1-10	**10-20**	**20-30**	**1-10**	**10-20**	**20-30**
	High irradiance†					
NTC	8.8c§*a*¶	14.3a*a*	9.6a*a*	379.6b*a*	506.4a*a*	297.2a*a*
Ax6548	11.5b*a*	11.7b*a*	8.3ab*a*	391.3b*a*	391.8b*a*	288.6a*a*
Ax6549	14.6a*a*	8.5c*a*	5.8b*a*	581.3a*a*	302.3c*a*	215.6a*a*
	Reduced irradiance‡					
NTC	4.2ab*b*	6.3a*b*	5.7a*b*	227.4ab*b*	346.3a*b*	281.4a*a*
Ax6548	6.3a*b*	5.3a*b*	5.3a*b*	292.3a*b*	273.4ab*b*	279.5a*a*
Ax6549	3.7b*b*	4.3a*b*	3.5a*a*	188.9b*b*	227.76b*a*	188.9a*a*

† 20 h photoperiod of 500 µmol photons m^{-2} s^{-1}

‡20 h photoperiod of 175 µmol photons m^{-2} s^{-1}

§ Within columns means followed by the same letter are not significantly different at P=0.05.

¶ Letters in italic indicate differences between the light treatments for one genotype.

Table 4.2: Net assimilation rate (NAR) expressed per unit leaf area and unit leaf mass of nontransformed control (NTC), Ax6548, and Ax6549 (n=8) calculated for day 1 to 10, 10 to 20, and 20 to 30 periods of July study, for plants grown under high irradiance and reduced irradiance treatments.

Line	RWR [g g^{-1}]				SWR [g g^{-1}]				LWR [g g^{-1}]			
	1	**10**	**20**	**30**	**1**	**10**	**20**	**30**	**1**	**10**	**20**	**30**
						High Irradiance†						
NTC	0.53a§*a*¶	0.28b*a*	0.26b*a*	0.26b*a*	0.29b*a*	0.37a*a*	0.47a*a*	0.52a*a*	0.18b*a*	0.35a*a*	0.27bb	0.22b*b*
Ax6548	0.54a*a*	0.30b*a*	0.25b*a*	0.34a*a*	0.25c*a*	0.35a*a*	0.40b*a*	0.40b*b*	0.21a*a*	0.35a*a*	0.35a*b*	0.26a*b*
Ax6549	0.40b*a*	0.39a*a*	0.38a*a*	0.38a*a*	0.39a*a*	0.38a*a*	0.37b*a*	0.40b*a*	0.21a*a*	0.23b*b*	0.25b*b*	0.22b*b*
						Reduced irradiance‡						
NTC	0.53a*a*	0.24b*a*	0.18b*b*	0.23a*a*	0.29b*a*	0.38a*a*	0.47a*a*	0.50a*a*	0.18b*a*	0.38a*a*	0.35b*a*	0.27b*a*
Ax6548	0.54a*a*	0.31a*a*	0.22ab*a*	0.21a*b*	0.25c*a*	0.33b*a*	0.38b*a*	0.47ab*a*	0.21a*a*	0.36a*a*	0.40a*a*	0.32a*a*
Ax6549	0.40b*a*	0.26b*b*	0.24a*b*	0.24a*b*	0.39a*a*	0.39a*a*	0.37b*a*	0.43b*a*	0.21a*a*	0.35a*a*	0.39a*a*	0.33a*a*

† 20 h photoperiod of 500 µmol photons m^{-2} s^{-1}

‡20 h photoperiod of 175 µmol photons m^{-2} s^{-1}

§ Within columns means followed by the same letter are not significantly different at P=0.05.

¶ Letters in italic indicate differences between the light treatments for one genotype.

Table 4.3: Root weight ratio (RWR), shoot weight ratio (SWR), and leaf weight ratio (LWR) of nontransformed control (NTC), Ax6548, and Ax6549 (n=8) grown under high irradiance and reduced irradiance, measured on day 0, 10, 20, and 30 of July study.

Line	**LAR** [m^2 g^{-1}]				**SLA** [m^2 g^{-1}]				**LW** [mm]			
	1	**10**	**20**	**30**	**1**	**10**	**20**	**30**	**1**	**10**	**20**	**30**
	High irradiance†											
NTC	0.009ab§*a*¶	0.015a*b*	0.008b*b*	0.007a*b*	0.050a*a*	0.041a*b*	0.032a*b*	0.032a*b*	1.5b*a*	2.1b*a*	2.4b*b*	3.0b*a*
Ax6548	0.008b*a*	0.012b*b*	0.012a*b*	0.009a*b*	0.038b*a*	0.033b*b*	0.034a*b*	0.036a*b*	1.0c*a*	1.6c*b*	2.2c*a*	2.7c*a*
Ax6549	0.010a*a*	0.009c*b*	0.009b*b*	0.008a*b*	0.047a*a*	0.037ab*b*	0.036a*b*	0.037a*b*	2.0a*a*	2.3a*b*	2.7a*b*	3.2a*a*
	Reduced irradiance‡											
NTC	0.009ab*a*	0.021a*a*	0.020aa	0.014b*a*	0.050a*a*	0.056a*a*	0.058a*a*	0.051a*a*	1.5b*a*	1.9b*b*	2.6b*a*	2.8b*b*
Ax6548	0.008b*a*	0.018b*a*	0.021a*a*	0.019a*a*	0.038b*a*	0.051a*a*	0.052b*a*	0.056a*a*	1.0c*a*	1.7c*a*	2.3c*a*	2.7c*a*
Ax6549	0.010a*a*	0.019b*a*	0.021a*a*	0.019a*a*	0.047a*a*	0.054a*a*	.0053ab*a*	0.056a*a*	2.0a*a*	2.5a*a*	3.0a*a*	3.2a*a*

† 20 h photoperiod of 500 µmol photons m^{-2} s^{-1}

‡20 h photoperiod of 175 µmol photons m^{-2} s^{-1}

§ Within columns means followed by the same letter are not significantly different at P=0.05.

¶ Letters in italic indicate differences between the light treatments for one genotype.

Table 4.4: Leaf area ratio (LAR), specific leaf area (SLA), and leaf width (LW) of nontransformed control (NTC), Ax6548, and Ax6549 (n=8) grown under high irradiance and reduced irradiance treatments, calculated for day 0, 10, 20, and 30 of July study.

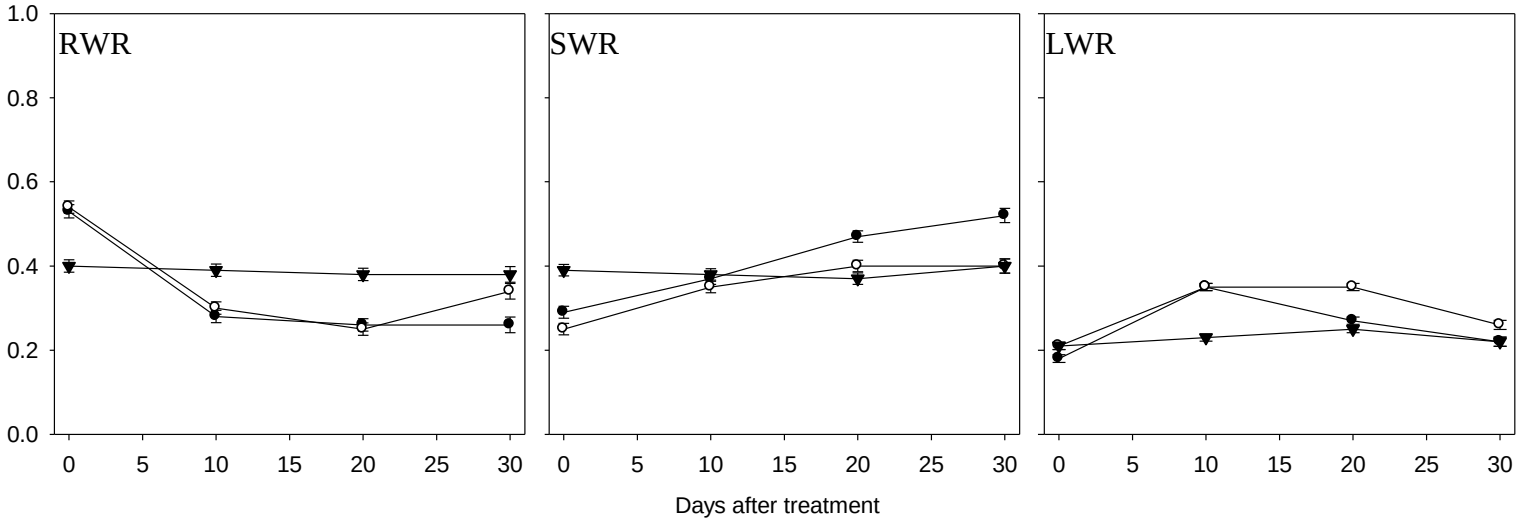

Fig. 4.1: Root weight ratio (RWR), stem weight ratio (SWR), and leaf weight ratio (LWR) of nontransformed control (NTC) (black circles), Ax6548 (white circles), and Ax6549 (black triangles) (n=8) grown under high irradiance (HI) during July study. Error bars designate standard error.

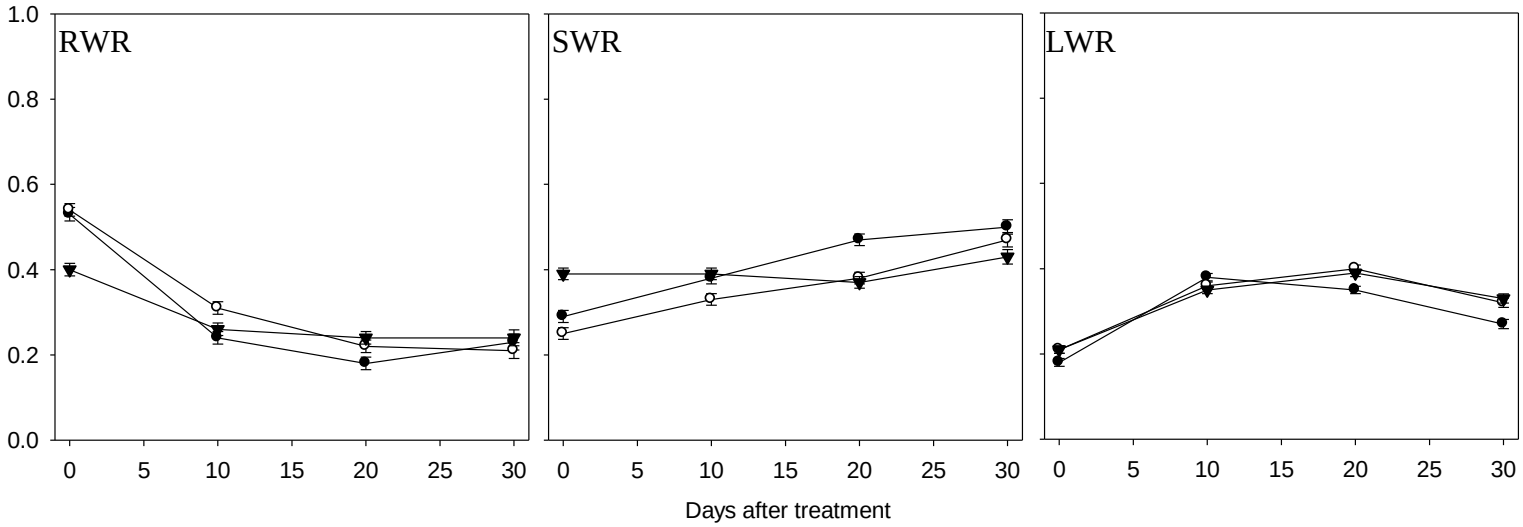

Fig. 4.2: Root weight ratio (RWR), stem weight ratio (SWR), and leaf weight ratio (LWR) of nontransformed control (NTC) (black circles), Ax6548 (white circles), and Ax6549 (black triangles) (n=8) grown under reduced irradiance (RI) during July study. Error bars designate standard error.

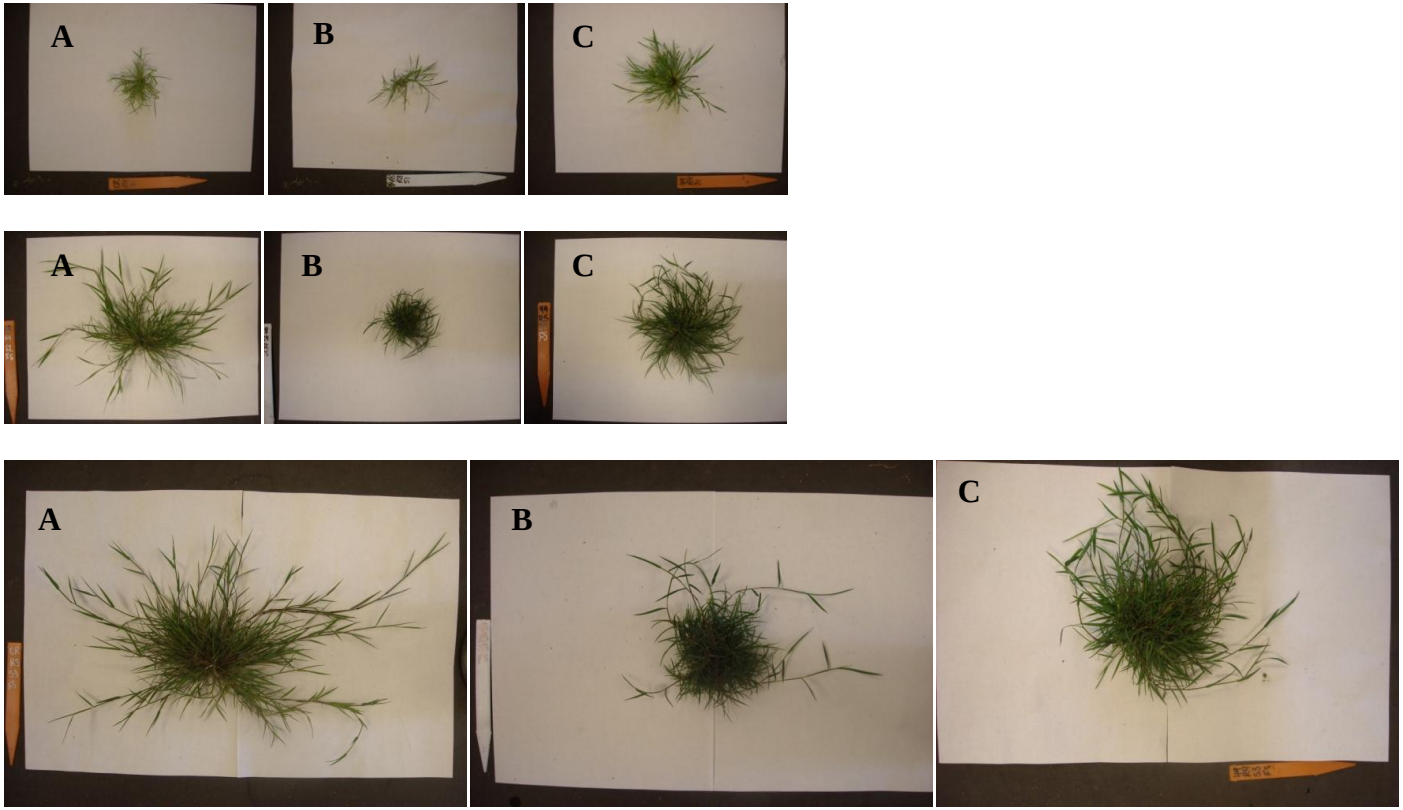

Fig. 4.3: Nontransformed control (A), Ax6548 (B), and Ax6549 (C) grown under high irradiance on day 1, 10, and 20 (from the top to the bottom) of July study.

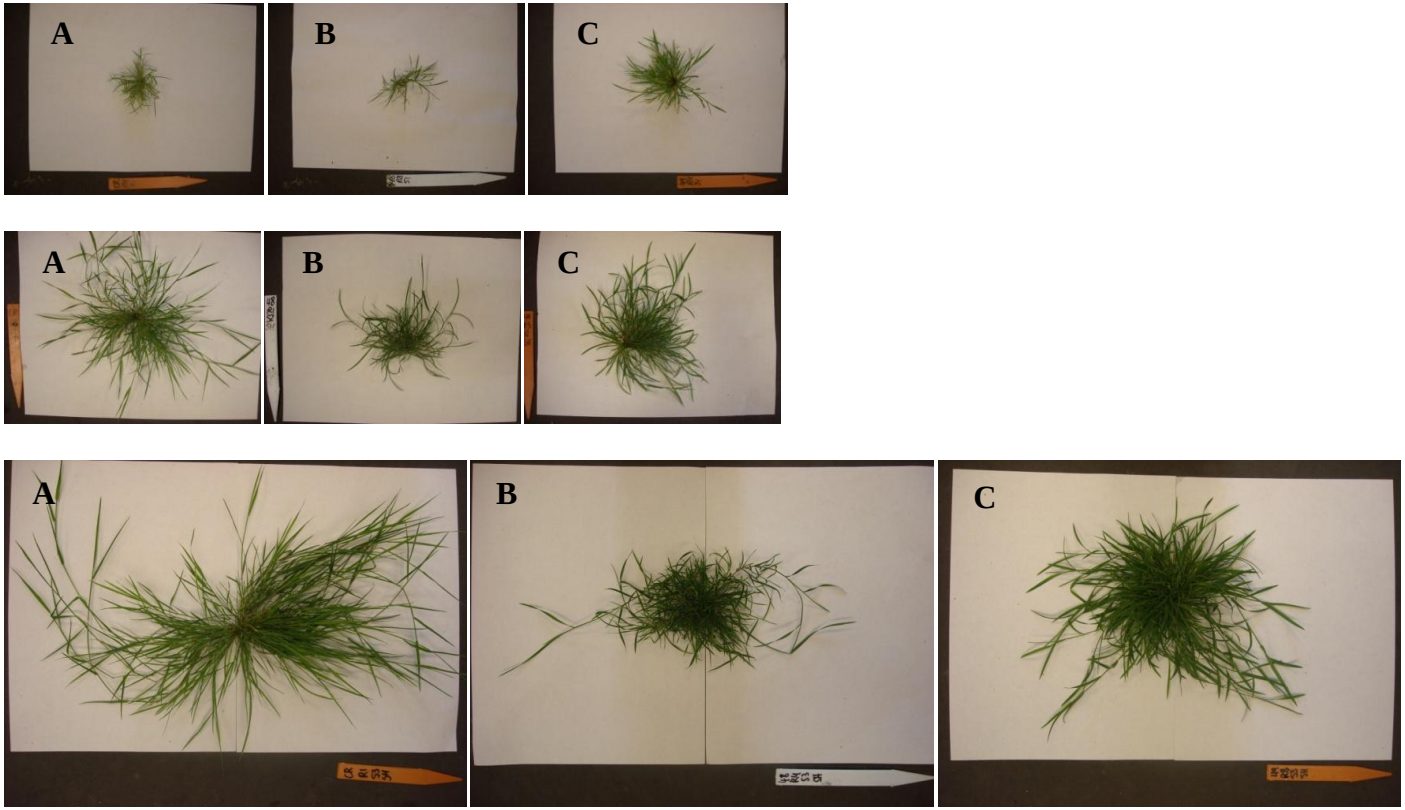

Fig. 4.4: Nontransformed control (A), Ax6548 (B), and Ax6549 (C) grown under reduced irradiance on day 1, 10, and 20 (from the top to the bottom) of July study.

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APPENDIX: GROWTH ANALYSIS DATA FROM JUNE STUDY

Line	**CGR** [mg d^{-1}]			**RGR** [mg g^{-1} d^{-1}]		
	1-10	**10-20**	**20-30**	**1-10**	**10-20**	**20-30**
	High irradiance†					
NTC	271.2a§*a*¶	365.5a*a*	497.0b*a*	118.2a*a*	50.4 ab*a*	41.6b*a*
Ax6548	125.9b*a*	314.4a*a*	1678.4a*a*	93.2b*a*	67.5a*a*	112.0a*b*
Ax6549	155.7b*a*	211.4b*a*	1794.8a*a*	83.5b*a*	44.6b*a*	129.2a*a*
	Reduced irradiance‡					
NTC	130.5a*b*	317.0a*a*	421.2b*a*	84.5a*b*	85.8a*b*	53.8b*a*
Ax6548	69.8ab*a*	127.7b*b*	1318.4a*b*	67.6a*b*	59.6b*a*	146.0a*a*
Ax6549	31.8b*b*	99.4b*b*	271.7c*b*	31.5b*b*	56.0b*a*	82.2b*b*

† 20 h photoperiod of 500 µmol photons m^{-2} s^{-1}

‡20 h photoperiod of 175 µmol photons m^{-2} s^{-1}

§ Within columns means followed by the same letter are not significantly different at P=0.05.

¶ Letters in italic indicate differences between the light treatments for one genotype.

Table A.1: Crop growth rate (CGR) and relative growth rate (RGR) of nontransformed control (NTC), Ax6548, and Ax6549 (n=8) calculated for day 1 to 10, 10 to 20, and 20 to 30 periods of June study, for plants grown under high irradiance and reduced irradiance treatments.

Line	**NAR** [g m^{-2} d^{-1}]			**NAR** [mg g^{-1} d^{-1}]		
	1-10	**10-20**	**20-30**	**1-10**	**10-20**	**20-30**
	High irradiance†					
NTC	5.7a‡*a*¶	3.5a*a*	4.5c*a*	391.0a*a*	222.3a*a*	215.0c*a*
Ax6548	5.2a*a*	5.1a*a*	11.0b*a*	268.2b*a*	244.6a*a*	409.3b*a*
Ax6549	6.0a*a*	4.3a*a*	14.9a*a*	275.0b*a*	185.8a*a*	528.3a*a*
	Reduced irradiance‡					
NTC	3.1a*b*	3.3a*a*	3.1b*a*	225.3a*b*	256.4a*a*	210.3b*a*
Ax6548	2.9a*b*	2.8a*b*	8.3a*b*	166.3ab*b*	168.3b*b*	462.4a*a*
Ax6549	1.7a*b*	2.7a*b*	4.8b*b*	85.38b*b*	160.1b*a*	283.0b*b*

† 20 h photoperiod of 500 µmol photons m^{-2} s^{-1}

‡20 h photoperiod of 175 µmol photons m^{-2} s^{-1}

§ Within columns means followed by the same letter are not significantly different at P=0.05.

¶ Letters in italic indicate differences between the light treatments for one genotype.

Table A.2: Net assimilation rate (NAR) expressed per unit leaf area and unit leaf mass of nontransformed control (NTC), Ax6548, and Ax6549 (n=8) calculated for day 1 to 10, 10 to 20, and 20 to 30 periods of June study, for plants grown under high irradiance and reduced irradiance treatments.

Line	RWR [g g^{-1}]				SWR [g g^{-1}]				LWR [g g^{-1}]			
	1	**10**	**20**	**30**	**1**	**10**	**20**	**30**	**1**	**10**	**20**	**30**
	High Irradiance†											
NTC	0.24a§*a*¶	0.22b*a*	0.24c*a*	0.26b*a*	0.42b*a*	0.50a*a*	0.57a*a*	0.55a*a*	0.34b*a*	0.28b*b*	0.20b*b*	0.19b*b*
Ax6548	0.23a*a*	0.24b*a*	0.31a*a*	0.32a*a*	0.39c*a*	0.43b*a*	0.45c*b*	0.38b*a*	0.38a*a*	0.33a*b*	0.24a*b*	0.30a*a*
Ax6549	0.22a*a*	0.26a*a*	0.28b*a*	0.32a*a*	0.46a*a*	0.44b*a*	0.52b*a*	0.41b*b*	0.32b*a*	0.30b*b*	0.20b*b*	0.27a*a*
	Reduced irradiance‡											
NTC	0.24a*a*	0.15a*b*	0.13b*b*	0.19b*b*	0.42b*a*	0.43a*b*	0.59a*a*	0.58a*a*	0.34b*a*	0.41a*a*	0.28b*a*	0.24c*a*
Ax6548	0.23a*a*	0.16a*b*	0.20a*b*	0.31a*a*	0.39c*a*	0.42a*a*	0.50b*a*	0.37c*a*	0.38a*a*	0.43a*a*	0.30ab*a*	0.33a*a*
Ax6549	0.22a*a*	0.15a*b*	0.20a*b*	0.20b*b*	0.46a*a*	0.43a*a*	0.49b*a*	0.53b*a*	0.32b*a*	0.42a*a*	0.32a*a*	0.27b*a*

† 20 h photoperiod of 500 µmol photons m^{-2} s^{-1}

‡20 h photoperiod of 175 µmol photons m^{-2} s^{-1}

§ Within columns means followed by the same letter are not significantly different at P=0.05.

¶ Letters in italic indicate differences between the light treatments for one genotype.

Table A.3: Root weight ratio (RWR), shoot weight ratio (SWR), and leaf weight ratio (LWR) of nontransformed control (NTC), Ax6548, and Ax6549 (n=8) grown under high irradiance and reduced irradiance, measured on day 0, 10, 20, and 30 of June study.

Line	**LAR** [m^2 g^{-1}]				**SLA** [m^2 g^{-1}]			
	1	**10**	**20**	**30**	**1**	**10**	**20**	**30**
	High irradiance†							
NTC	0.028a§*a*¶	0.018a*b*	0.012a*b*	0.008a*b*	0.081a*a*	0.063a*b*	0.064a*b*	0.041a*b*
Ax6548	0.021b*a*	0.017a*b*	0.012ab*b*	0.010a*b*	0.054b*a*	0.051b*b*	0.047b*b*	0.035a*b*
Ax6549	0.015c*a*	0.013b*b*	0.009b*b*	0.009a*b*	0.047b*a*	0.045b*b*	0.043b*b*	0.033a*b*
	Reduced irradiance‡							
NTC	0.028a*a*	0.030a*a*	0.023a*a*	0.013a*a*	0.081a*a*	0.071aa	0.081a*a*	0.057a*a*
Ax6548	0.021b*a*	0.026b*a*	0.018b*a*	0.017a*a*	0.054b*a*	0.060b*a*	0.060b*a*	0.054a*a*
Ax6549	0.015c*a*	0.023b*a*	0.021ab*a*	0.013a*a*	0.047b*a*	0.055b*a*	0.065b*a*	0.055a*a*

† 20 h photoperiod of 500 µmol photons m^{-2} s^{-1}

‡20 h photoperiod of 175 µmol photons m^{-2} s^{-1}

§ Within columns means followed by the same letter are not significantly different at P=0.05.

¶ Letters in italic indicate differences between the light treatments for one genotype.

Table A.4: Leaf area ratio (LAR) and specific leaf area (SLA) of nontransformed control (NTC), Ax6548, and Ax6549 (n=8) grown under high irradiance and reduced irradiance treatments, calculated for day 0, 10, 20, and 30 of June study.

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