A holistic analysis of photosynthetic acclimation to shade in C4 grass (Cynodon dactylon (L.) Pers.)

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Research Article

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Abstract

Background: Lack of light in shady environment is the key factor affecting the growth and development of turf plants. However, it is shade avoidance rather than shade tolerance that has received more attention in past decades. In this study, we investigated the photosynthetic metabolisms of Bermuda grass exposed to deepening shade, aiming to provide a holistic perspective for understanding the shade acclimation strategies of C4 turfgrass.

Results: The adjustments of pigment-proteins, photosynthetic electron transport and its coupling of carbon and nitrogen assimilation, ROS-scavenging enzyme activity in shade surroundings were detected. Mild shade enriched Chl b and LHC transcripts, while Chl a, carotenoids and photosynthetic electron transfer beyond Q_A^- (ET_0/RC, φE_0, Ψ_0) were enhanced by severe shade. In addition, differential impacts of shade on leaf and root were shown. Soluble sugar deficiency varied between the two, due shade reduced *SPS, SUT1* while up-regulated *BAM*. Besides, Shading weakened the transcriptional level of genes involving in nitrogen assimilation (e.g. *NR*) and SOD, POD, CAT enzyme activities in leaf, but improved them in root.

Conclusions: As the shade deepened, extensive changes had taken place in light energy conversion and photosynthetic metabolism process along the axis of electron transport chain. This study provided a theoretical basis for the photosynthetic acclimation of C4 grass to shade tolerance.

Background

Light is essential to the growth and development of plants. Not only is it a source of solar energy for photosynthesis, it also acts as an environmental signal orchestrating morphological and physiological trade-offs throughout the whole life cycle (Kami et al, 2010). However, shade circumstances were common barrier in the process of agricultural production (Pierik and Ballaré, 2020) and urban greening (Richnau et al, 2016). The lower niche plants in the intercropping or vertical planting system receives only limited light (Qiao et al, 2019; Li et al, 2007), especially ground covers and lawns (Xu et al, 2010; Jiang et al, 2004; Bell et al, 2000).

Filtered by the upper plants, the light intensity at the bottom is reduced. Red (R, λ = 600–700 nm) and blue (B, λ = 400 ~ 500 nm) light are largely trapped by chloroplasts, while far red light (FR, λ = 700 ~ 800 nm) is partially preserved by reflection of surroundings (Vandenbussche et al, 2005; Bell et al, 2000). The disproportional decrease creates depressed R:FR ratio and low B, which is perceived by photoreceptors as shade signal, and results in two contrasting strategies to cope with, shade avoiding or shade tolerant (Fiorucci et al, 2017). Manifested by rapid elongation of stems and petioles, accelerated flowering, the shade-avoidance syndrome (SAS) had been substantially studied (Fernández-Milmanda and Ballaré, 2021; Fraser et al, 2016; Franklin, 2008; Cho et al, 2007). However, it only works on plants have similar heights which settle in open habitats. For understories, elongation is no longer an option to escape from the shade due to the great disparity in height (Gommers et al, 2013). They prefer to use tactics of shade

tolerance by suppressing SAS and directing resources toward to optimize photosynthesis and strengthen physical defense rather than in vain elongation (Gamage, 2011; Niinemets and Valladares, 2004; Morgan and Smith, 1979). To date, the knowledge of shade tolerance still unstructured (Gommers et al, 2013, Valladares and Niinemets, 2008), especially in understory herbs (Klimeš et al, 2021; Bierzychudek, 1982), which was largely absent.

Given that the shading perception and response are partly overlapped in shade and non-shade plants (Evans and Poorter, 2001; Melis and Harvey, 1981). Charlotte (2013) assumed that the molecular regulatory components were shared between shade tolerance and shade avoidance. It is the different signal transduction path that leads to the difference in the choice of strategy. In the molecular cascades of shade response, the PIFs (Phytochrome-Interacting Factors, PIF4, PIF5, PIF7) are the core signaling hub that coordinates majority of the downstream events (Pierik and Ballaré, 2020; Ballaré and Pierik, 2017). At low R:FR ratio, the degradation of PIFs is decelerated by impaired phosphorylation through the inactivation of phyB (Hornitschek et al, 2012). The accumulation of PIFs pool, particularly PIF7 (Pantazopoulou et al, 2017; Li et al, 2012), activates the auxin network and causes elongation (Iglesias et al, 2018). In addition, the attenuation of blue light was perceived by cryptochromes (cry1 and cry2), and the inactivated cry also enhanced the abundance of PIF (PIF4, PIF5) (Pedmale et al, 2016; Keller et al, 2011). Importantly, the activity of PIFs are balanced by inhibitors in a feedback modulated manner, including HFR1, PAR1, PAR2 (Buti et al, 2020) and DELLA protein (Djakovic-Petrovic et al, 2007). The antagonistic factors that inhibit the regulatory pathway of SAS may indicate the direction of elucidating the shade tolerance mechanisms (Vandenbussche et al, 2005).

The acclimations to low light also included the adjustment of leaf anatomical structure and chloroplast ultrastructure from the whole organism to the cell. A higher specific leaf area (Evans et al, 2001), larger proportion of spongy tissue (Vogelmann et al, 1993), and greater grana thylakoid stacking level (Niinemets, 2007) were showed in shade leaves when compared to sun leaves. Moreover, the capacity of electron transport and metabolisms coupling with light reactions synchronizes with the given environmental conditions (Morales and Kaiser, 2020; Gjindali et al, 2021). A new balance between the photosynthetic electron transport (Kono and Terashima, 2014), the Calvin-Benson cycle (Nikkanen and Rintamäki, 2019), nitrogen assimilation (Stitt et al, 2002) and reactive oxygen production (Shikanai and Yamamoto, 2017) wound be established, due to the more furious competition of photoelectron or reducing forces under limited light.

To match the low irradiance environment, post-translational regulation is usually rapid and immediate, which mainly embody at the activity of metabolic enzymes. They were modified by protein phosphorylation (e.g. Nitrate reductase, EC:1.7.1.1) (Su et al, 1996), sulfhydryl reduction (e.g. Rubisco EC:4.1.1.39, fructose-1,6-bisphosphatase EC:3.1.3.11, sedoheptulose-1,7- bisphosphatase EC:3.1.3.37) (Montrichard et al, 2009) and ionic prosthetic group (e.g. Mg^{2+}, H^{+}) (Armbruster et al, 2014). While the sustained changes in transcription level and protein abundance make important contributions to long-term acclimation. The concentration of enzymes in Calvin-Benson cycle was observed to shift with light intensity (Miller et al, 2017). PSII/PSI ratio and LHCII increased when exposed to low light (Bailey et al,

2001). The transcription level of genes encoding nitrate transporter (e.g. *NRT2.1*) and nitrate reductase (e.g. *NIA2*) could be directly regulated by HY5 or indirectly regulated by SWEET1/2-mediated sucrose signaling (Sakuraba and Yanagisawa, 2018). The abundance of HY5 was governed by the COP1/SPA complex (Gangappa and Botto, 2016), a downstream key regulatory factor of Phy or Cry, to coordinate nutrient acquisition and utilization with fluctuating light. Shading acclimations mentioned above are found in C3 photosynthesis, which has been extensively studied over the past several decades, while related research in C4 was almost blank.

Bermudagrass (Cynodon dactylon (L.) Pers.) is typical warm-season (C4) perennial grass which belongs to the NAD-ME biochemical subtype (Carmo-Silva et al, 2008; Cui F et al, 2021). It is widely used as turfgrass or forage dues to its excellent resistance of abiotic stresses, however it is sensitive to shade (Baldwin et al, 2008). Characterized by the CO2-concentrating mechanism, C4 plants possess ecological dominance in a warm, high-light environment (Edwards et al, 2010). But lower plasticity and higher energy consumption result in limited survival when C4 plant was exposed to shade circumstance (Sage and McKown, 2006; Sonawane et al, 2018) because of the specialized adaptation on tissues (Kranz anatomy) and carbon metabolism (the overcycling of CO2). Especially in the NAD-ME type, more N fraction is invested in Rubisco instead of the light-harvesting antenna under shade (Ghannoum et al, 2005). It leads to a greater decrease of enzyme activity in C3 cycle (Rubisco) compared with C4 cycle (PEPC, Phosphoenolpyruvate carboxylase, EC:4.1.1.31), which was further give rise to C3/C4 uncoupling (Bellasio and Griffiths, 2014a,b).

To investigate the long-term photosynthetic acclimation to shade of C4 grass, bermudagrass was covered with shading net for one week. Taking the photosynthetic electron transport as the core, we linked ROS metabolism with C/N assimilation to track the process of energy absorption, transport and utilization on the thylakoid membrane, comprehensively analyzed the shade-tolerant adaptability of bermudagrass from a holistic perspective.

Results

Changes of photosynthetic pigments under shading conditions

A linear change of photosynthetic pigment content had been observed when bermudagrass against the shading treatment. The total amount of pigment decreased with deepening shade, which predominantly due to the dramatic decline of chlorophyll b (Fig. 1A**)**. The density of chlorophyll b in group E was almost half that of group B. It was worth noting that chlorophyll b increased considerably in group B compared with the control (group A), and then brought down the ratio of chla to chlb. However, carotenoid content increased significantly, from 1.63% in group B to 12.27% in group E of the total. There was also an significant increase in chlorophyll a content.

Further detection of pigment biosynthesis gene expression level in selected three groups shed light on the reason of pigments change. The expression level of *HEMA*, which coded glutamyl-tRNA reductase catalyzing the first step of chlorophyll biosynthesis, was gradually down-regulated with the deepening of shade (Fig. 1B). This may had led to a decrease in the total amount of chlorophyll. Besides, the abundance of light-harvesting complex (*LHCA2, LHCB2*) transcripts were accumulated under low shading and perished under high shading (Fig. 1D). The increased LHC may be result in the decrease of chla/chlb in group B, because chlorophyll b was mainly bound to the peripheral antenna instead of the core complex in two photosystems. In the carotenoid biosynthesis pathway, the *PSY* level was sharply up-regulated by shading while the *PDS* level was firstly increased and then decreased (Fig. 1C). This may account for the rise in carotenoid levels. Besides, the transcriptional level of *PORA* was raised 3.9 times in group E compared to control (Fig. 1B), which may intent to prevent the burst of ROS caused by the accumulation of phototoxic intermediates when sudden exposure to light.

The electron transfer beyond Q was enhanced with shade

Chlorophyll a fluorescence transient curves (O-J-I-P) were reshaped by increased shade (Fig. 2A). Fv was lifted up from group A to D, and dropped in E (Fig. 2B, Table 1). It implied the photosynthetic adaptation pattern was moved from shade resistance to shade tolerance. We artificially divide the curve into two sections according to the number of Q_A^- reduction, O-J and J-P phase. One obvious change in O-J phase was that the initial fluorescence (F_0) increased (A to C) with darkening surroundings (Fig. 2C, Table 1). This may indicate an increase in light-harvesting capacity, since LHC transcriptional levels and photosynthetic pigments are correspondingly increased in Fig. 1A.

Be observed the variation of J-P phase is greater than that of O-J phase (Fig. 2A), we emphatically analyzed the parameters of electron transfer beyond Q_A^-. ET_0/RC (electron transport flux further than Q_A^- per RC) was significantly rose in D and E treatment compared with the control.

Table 1
The variation of fluorescence transient parameters of Bermudagrass under shade

	A	B	C	D	E	Definitions
Data extracted from the recorded fluorescence transient OJIP						
F_0	0.61b	0.64ab	0.67a	0.66a	0.67a	Fluorescence at time 20 μs after onset of actinic illumination
F_m	2.28c	2.31bc	2.36b	2.48a	2.32bc	Maximal recorded fluorescence intensity, at the peak P of OJIP
F_k	1.41b	1.45ab	1.50a	1.51a	1.25c	Fluorescence value at 300 μs
F_j	1.63a	1.64a	1.67a	1.69a	1.44b	Fluorescence value at the J-step (2 ms) of OJIP
F_i	2.00c	2.08b	2.12b	2.21a	1.97c	Fluorescence value at the I-step (30 ms) of OJIP
Fluorescence parameters derived from the extracted data						
Area	49.35b	48.09b	49.15b	52.59b	58.74a	Total complementary area between the fluorescence induction curve and F = F_m
F_v	1.66b	1.67b	1.68b	1.82a	1.65b	Maximal variable fluorescence
V_k	0.48a	0.49a	0.49a	0.47a	0.35b	Relative variable fluorescence at k step
V_j	0.61a	0.60a	0.60a	0.55b	0.46c	Relative variable fluorescence at J step
V_i	0.84b	0.86a	0.86ab	0.85ab	0.79c	Relative variable fluorescence at I step
M_0	1.91a	1.95a	1.97a	1.87a	1.39b	Approximated initial slope (in ms − 1) of the fluorescence transient
S_m	29.70b	28.76b	29.18b	28.96b	35.69a	Normalized total complementary area above the O-J-I-P transient
Ss	0.32ab	0.31b	0.30b	0.30b	0.33a	Normalized total complementary area corresponding only to the O-J phase
N	93.06b	93.39b	95.82b	98.02b	106.59a	Turnover number: number of Q_A reduction events between time 0 and tF_m

Note: The mean values from five biological replicates were listed in Table 1, followed by the letters showing statistical differences. ANOVA with Student-Newman-Keuls ($P < 0.05$) was conducted in present research.

	A	B	C	D	E	Definitions
Data extracted from the recorded fluorescence transient OJIP						
Quantum yields and efficiencies						
φP_0	0.73a	0.72a	0.71a	0.73a	0.71a	Maximum quantum yield of primary photochemistry (at t = 0)
Ψ_0	0.39c	0.40c	0.40c	0.45b	0.54a	Efficiency/probability that an electron moves further than Q_A-
φE_0	0.28c	0.29c	0.28c	0.33b	0.38a	Quantum yield of electron transport (at t = 0)
φD_0	0.27a	0.48a	0.29a	0.27a	0.27a	Quantum yield (at t = 0) of energy dissipation (at t = 0)
φR_0	0.12b	0.10c	0.10c	0.11bc	0.15a	Quantum yield for reduction of end electron acceptors at the PSI acceptor side
δR_0	0.42a	0.34b	0.36b	0.34b	0.39a	Efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (RE)
γ RC	0.19a	0.18a	0.18a	0.13b	0.19a	Probability that a PSII Chl molecule functions as RC
RC/ABS	0.22a	0.22a	0.22a	0.22a	0.24a	QA-reducing RCs per PSII antenna Chl (reciprocal of ABS/RC)
Specific energy fluxes (per Q_A-reducing PSII reaction center/RC)						
ABS/RC	4.29a	4.49a	4.60a	4.64a	4.22a	Absorption flux (of antenna Chls) per RC (at t = 0)
TR_0/RC	3.13ab	3.25ab	3.28ab	3.39a	2.99b	Trapping flux (leading to Q_A reduction) per RC (at t = 0)
ET_0/RC	1.22b	1.30b	1.31b	1.52a	1.61a	Electron transport flux (further than Q_A−) per RC (at t = 0)
DI_0/RC	1.16a	1.24a	1.32a	1.25a	1.22a	Dissipated energy flux per RC (at t = 0)
Phenomenological energy fluxes (per excited cross section/CS)						
RC/Cs_0	0.14a	0.19a	0.15a	0.14a	0.16a	Density of RCs (QA-reducing PSII reaction centers) (at t = 0)

Note: The mean values from five biological replicates were listed in Table 1, followed by the letters showing statistical differences. ANOVA with Student-Newman-Keuls ($P < 0.05$) was conducted in present research.

	A	B	C	D	E	Definitions
Data extracted from the recorded fluorescence transient OJIP						
ABS/Cs_0	0.61a	0.85a	0.67a	0.66a	0.67a	Absorption flux per CS, approximated by F_0 (at t = 0)
$TR0/Cs_0$	0.45a	0.61a	0.48a	0.49a	0.48a	Trapped energy flux per CS (at t = 0)
$ET0/Cs_0$	0.18a	0.24a	0.19a	0.22a	0.26a	Electron transport flux per CS (at t = 0)
$DI0/Cs_0$	0.17a	0.91a	0.19a	0.18a	0.20a	Dissipated energy flux per CS (at t = 0)
Performance indexes						
PI_{ABS}	0.41c	0.39c	0.36c	0.49b	0.67a	Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
PI_{Total}	0.30b	0.20b	0.20b	0.25b	0.44a	Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors
PI_{CS}	0.25b	0.33ab	0.24b	0.32ab	0.45a	Performance index on cross section basis
Note: The mean values from five biological replicates were listed in Table 1, followed by the letters showing statistical differences. ANOVA with Student-Newman-Keuls ($P < 0.05$) was conducted in present research.						

φE_0 (efficiency/probability that an electron moves further than Q_A^-), Ψ0 (quantum yield of electron transport),S_m and N (the pool size of the electron acceptor beyond Q_A^-), and PI_{ABS} (Performance index for energy conservation from exciton to the reduction of intersystem electron acceptors) appeared a similar performance (Fig. 3A, B, Table 1). All results indicated that electron transport was enhanced with deeper shade stress. In addition, the transcriptional level of PGR5/PGR5L, a key component of cyclic electron transport, was induced by shade signal in group A to C (Fig. 2D). It implied the electron after Q_A^- was pulled by the cyclic electron transport.

Besides, φP_0, ABS/RC and TR_0/RC showed no significant change with shade, while ABS/Cs_0, TR_0/Cs_0, RC/Cs_0 raised in group B (Table 1). It suggested that shade did not affect the absorption and trapping of light energy per unit RC, but increased the number of RCs per unit cross-sectional area, which resulted from photosynthetic pigment enrichment.

The accumulation and transport of carbohydrates were weakened under shade

In present research, soluble sugar content in leaves showed a U-shaped curve with increasing shade and reached the minimum in group C (Fig. 4A). However, the content in roots decreased linearly (Fig. 4A). It speculate that the rise of soluble sugar content in D and E group may be due to starch degradation, but even so, the supply of carbohydrate in roots was still limited. To explore the reasons behind, we examined the enzyme activity or transcription levels of key components involved in the synthesis, transport, and decomposition of unstructured carbohydrates (sucrose and starch). Enzyme activity tests in vitro showed that Rubisco activity was significantly increased compared with PEPC (Phosphoenolpyruvate Carboxylase) (Fig. 4B). It may due to the protein level of Rubisco was enriched by shade. However, the accumulation of protein may not effectively improve enzyme activity because of its activity also received co-regulation of light. Instead, the accumulation of Rubisco increased material consumption although in the case of resource shortage. The transcript level of *TPT* (Triose Phosphate Translocator, providing sucrose synthesis precursors), *SPS* (Sucrose Phosphate Synthase, key rate-limiting enzyme in sucrose biosynthesis), *SUT1* (Sucrose Transporter, transporting sucrose to the sieve element-companion cell for phloem loading), *SS* (Sucrose Synthase, participating in the breakdown of sucrose) were all down-regulated with increasing shade (Fig. 4C). The impairment of sucrose metabolism in leaves probably resulted in the linear decrease of soluble sugar in roots, because sucrose was the main form of carbohydrate transport in plants. Meanwhile, we detected the up-regulation of *BAM* (Beta amylase) transcription levels (Fig. 4D). It indicated that the carbohydrate budget was in deficit and starch was requisitioned, which account for the rise of soluble sugar in D and E group.

The nitrogen assimilation adjustments of plant in shade surrounding

Carbon and nitrogen metabolism in plants was closely coupled and competitive, which constitutes the material basis of crop yield and quality respectively. The crude protein content (Per dry weight of leaf, a vital indicator of forage quality) increased under mild shade (Fig. 5A). Conversely, soluble protein content decreased in low shade and recovered with aggravating shade (Fig. 5A). It suggested the distribution of nitrogen in bermudagrass varied in different shade environments. Under slight shade, insoluble proteins was synthesized, probably the light-harvesting antennas. Under severe shade, the enrichment of Rubisco protein may be the main cause of the increase of soluble protein. Organic nitrogen was converted from inorganic nitrogen (NO_3^-, NH_4^+) by nitrogen assimilation. In present research, the transcriptional level of enzymes which associated with the process was detected. *FNRL2* (Ferredoxin-NADP reductase, leaf isozyme 2), *NR* (nitrate reductase), *NIR* (Ferredoxin–nitrite reductase) transcript level reduced gradually with deepening shading (Fig. 5B). It suggested the reduction of nitrate to ammonium was inhibited by shading in leaves. *FD* (Ferredoxin, chloroplastic) expression level increased under mild shade and then decreased with severe shade. Interestingly, *GS2* (Glutamine synthetase) and *Fd-GOGAT* (glutamine

oxoglutarate aminotransferase) transcript level had same performance as *FD* (Fig. 4B, C). It may be due to the ferredoxin provided reducing power for the latter two. However, *NR* and *GS1* level in root was elevated by shade (Fig. 5D). Taken together, we suggested that both the reduction force and carbon assimilation shortage, which caused by the diminution of light intensity, might be account for the displacement of nitrogen assimilation site. The roots were responsible for more nitrogen assimilation than the chloroplasts.

The antioxidant capacity of leaves was opposite to that of roots

In this study, we investigated the antioxidant capacity of the two tissues under shading in terms of transcription level and enzyme activity. The results showed that the changes of enzyme activities with light intensity were completely opposite between leaf and root (Fig. 6A, C). The SOD, POD, CAT enzyme activity in leaf showed a U-shaped curve with the decrease of light intensity and reached the minimum value in group C (Fig. 6A). Although three enzyme activities rose again in group D and E, it was still lower than the initial level. On the contrary, the changes of SOD, POD, CAT enzyme activity in roots showed a parabolic pattern with increasing shading degree, group C had the maximum value (Fig. 6C). However, the expression levels of genes encoding antioxidant enzymes increased first and then decreased with deepening shade in both leaves and roots (Fig. 6B, D). This may indicate the existence of post-transcriptional regulation of antioxidant enzymes.

Discussion

It is generally believed that shade tolerant plants have lower ratio of chlorophyll a/b and higher ratio of PSII/ PSI than sunny plants (Gommers et al, 2013). The adjustments of photosynthetic apparatus elevate the light harvesting capacity to meet the needs of surviving in a shady environment. Chlorophyll b plays an important role in regulating the antenna size of photosynthetic apparatus and maintaining stability of LHCII (Yamasato et al, 2005; Voitsekhovskaja and Tyutereva, 2015; Peng et al, 2018). In present research, the photosynthetic pigment biosynthesis and *LHC* transcriptional levels showed adaptive changes with the increase of shade degree (Fig. 1). The ratio of chlorophyll a/b and *LHC* transcripts level first rise then descended with decreasing light intensity, it demonstrates bermudagrass transform from active adaptation to passive tolerance for resisting deepening shade. While carotenoid content showed a continuous increase, changes in expression levels of related genes encoding enzymes in the pigment synthesis pathway account for it. Previous studies have shown that PIFs inhibits the expression of *HEMA* (Moon et al, 2008) and *PSY* (Toledo-Ortiz et al, 2010), however PAR1 prevents PIF1 from inhibiting PSY1 expression under shade (Bou-Torrent et al, 2015), thus may induce carotenoid accumulation. Moreover, shade leaves possess more grana thylakoids and higher ratio of PSII/ PSI (Oguchi et al, 2010). The high proportion of far-red light under shade conditions produces the dephosphorylation of LHCII, which makes the photosynthetic apparatus transform to state 1. However, the absorption efficiency of PSI for far-red light was higher than that of PSII, higher ratio of PSII/ PSI in leaves is beneficial to balance the light

energy absorption of two photosystems (Tikkanen et al, 2010). State transitions are considered as a way to maximize the efficiency of light harvesting at low light intensity (Conrad et al, 2005; Xu et al, 2015).

Sugars are not only energy sources and important components of structural substances in plants, but also a signal regulating the expression of related genes and enzyme activity (Kunz et al, 2014). Sucrose-non-fermenting-related-kinase1 (SnRK1) is a hub regulator involving in energy-signaling in higher plants (Elena and John, 2020), and respond to energy deficit signal in dark environments (Simon et al, 2018). It is participate in the sugar metabolism pathway by regulating the expression of genes related to sucrose synthesis, starch synthesis and degradation. SnRK1 inhibits the activities of sucrose phosphate synthase (SPS) and nitrate reductase (NR) at the post-transcriptional level (Polge and Thomas, 2007), and regulates sucrose synthase (SS) and α-amylase (α-AMY) at the transcriptional level (Sophie et al, 2003; Purcell et al, 1998). Otherwise, in HXK1-dependent sugar signal transduction pathway, HXK1 participates in the transcription repression of photosynthesis-related gene (such as Rubisco and LHC) by glucose (Jang et al, 1997; Cho et al, 2006). In a word, sugar signals in plants coordinate carbon/nitrogen metabolism and the expression of photosynthesis-related genes.

Light signals are also involved in carbon/nitrogen metabolism. It was previously reported the promoter activity of OsSPS1 and OsSPS11 is not regulated by sucrose, but by light and circadian clock (Yonekura et al, 2013). In the blue light signaling pathway mediated by cryptochrome 1A (CRY1A), HY5 directly binds to the promoter of starch degradation related genes (including BAM1, BAM3 and BAM8) to induce chloroplast starch degradation in tomato (Dong et al, 2020). Besides, HY5 can also binds to the promoter of NR and promote its transcription (Lee et al, 2007), it shows the crosstalk of the sugar signal and the light signal. In fact, NR, GS2 and GOGAT are frequently found to be light-induced with photosynthesis-related gene at same time (Jonassen et al, 2008). In the present study, the transcription levels of LHC, GS, GOGAT and SS changed synchronously, and negatively correlated with soluble sugar content in leaves (Fig. 1D, 4C, 5C). Meanwhile, SPS and NR transcripts in leaves were significantly down-regulated in shaded environment (Fig. 4C, 5B), it suggested that carbon and nitrogen metabolism was inhibited by shade. However, NR and GS in root raised with shade level, we hypothesize that roots are non-photosynthetic organs and not sensitive to light.

The source of reactive oxygen species in leaves was largely due to the excessive activation of photosynthetic electron transport in chloroplasts (Ivanov et al, 2003). Limited light energy input in low light conditions decreased the production of ROS in chloroplasts. ROS in roots mainly come from mitochondria (Khandelwal et al, 2008). Energy shortage caused by low sucrose import under low light was potentially responsible for the imbalance of ROS and antioxidant enzyme activity changes. Crosstalk between plant sugar signal and hormone signal was also exist (Soulaiman et al, 2018; Depaepe et al, 2021), and the hub regulator SnRK1 involved in the regulation of antioxidant enzyme (SOD, POD, CAT) activity and gene expression (Wang et al, 2020).

Conclusion

In present research, improvement in light-harvesting capacity (enriched photosynthetic pigments content and *LHC* transcripts), and in the photosynthetic electron transfer beyond Q_A^- (up-regulated ET_0/RC) was observed in Bermuda grass under mild and severe shade respectively. In addition, differential impacts of shade on leave and root were shown. Compared with root, the soluble sugar deficit in leave was filled, due to the up-regulation of genes related to starch degradation. Shade weakened the transcriptional level of genes involved in nitrogen assimilation and antioxidant enzyme activity in leave, while enhanced them in root. Orchestration of sugar, light and ROS signals may account for these adjustments in photosynthetic acclimation.

Methods

Plant Material

The plant material in this study was an herbage-type cultivar, 'Wrangler' Bermuda grass, from the grass resource germplasm nursery of Ludong University. To eliminate inter-individual differences of seeding, stolon originated from the mother plant was uniformly propagated. A special seedling-raising tube (5 cm diameter and 25 cm deep) fill of silver sand, which inhaled with half-strength Hoagland's solution (1/2 HS), was used as culture system. Covered with wet sand, node of stolon took root and developed intact seeding in controlled greenhouse for one month. Growth conditions were set as 24/20°C (day/night), relative humidity of 40%, natural light.

Treatment

In order to simulate the surroundings of understory herbaceous species, sunshade net (shading coefficient was 50%) with different layers was equipped to create a gradient in light intensity. By which, seeding of bermudagrass were divided into five groups as follows: (i) natural light (500 μmol m^{-2} s^{-1}) (A); (ii) covered with one layer of sunshade net (250 μmol m^{-2} s^{-1}) (B); (iii) covered with two layers of sunshade net (125 μmol m^{-2} s^{-1}) (C); (iv) covered with three layers of sunshade net (62.5 μmol m^{-2} s^{-1}) (D); (iv) covered with four layers of sunshade net (31.25 μmol m^{-2} s^{-1}) (E). The illumination intensity was separately recorded as in A, B, C, D, E. Each treatment had five duplications (tubes).

Photosynthetic pigment examination

0.1g leaves was collected and submerged in 5 ml dimethyl sulfoxide at 4^{o}C. After 24 hours in the dark, the absorbance of the extract at 645 nm, 663 nm, 440 nm were measured by ultraviolet spectrophotometer. The pigment content was calculated as described (Li et al, 2021).

Chlorophyll a Fluorescence Transient and the JIP-Test

To detect the organization and operating state of the PSII of bermudagrass in the shade, we conducted chlorophyll a fluorescence transient by Pulse-Amplitude-Modulated (PAM) Chlorophyll Fluorometer (PAM2500, Heinz Walz GmbH) to obtain multiphase rise curve (O-J-I-P). 30 min aphotic adaption for blades was essential until 2 s red saturated pulsed light (650 nm, 3500mmolm-2s-1) flashed. A leaf clip

assisted the optical fiber in irradiating the leaf surface vertically. Seedlings were kept in respectively light conditions except which being measured.

We further decomposed the O-J phase into W_k = (Ft − Fo) / (F_K−Fo) and ΔW_K=W_K treatment -W_K ref (“ref” is for natural light in A group) to shed light on details of the primary photochemical reaction procedure. In ΔW_K curve, the L-band (at about 150 μs) usually prompted the energetic connectivity among the PS II RC components. In ΔW_K curve, the L-band (at about 150 μs) showed energetic connectivity deficit (affluence) of the treatment group when it was positive (negative).

Basing on the theory of “energy flow” model, the JIP-Test converted abstract light energy conversion and transfer efficiency into numerical value (Strasser et al, 1995, 2000, 2004). In which, the recorded fluorescence singal at particular moment (0.2 ms, 2 ms, 30 ms) deduced a considerable information about the absorption (ABS) and trapping (TR_0) of light quantum, dissipation (DI_0) in light-harvesting antenna, electron transport (ET_0) though two optical systems, and reduction of end acceptors of PSI (RE_0). The parameters classified into four categories: (1) basic measured and calculated values (2) quantum yields and efficiencies; (3) specific energy fluxes and (4) performance indices. Each treatment had five duplications.

The assay of soluble sugar and protein

0.2 g chopped fresh leaves or roots were boiled in distilled water for 30 min. After filtration, the volume was constant to 25 ml. 0.5 ml filtrate was used to detect soluble sugar content according to phenol method with sucrose as the standard (Li et al, 2021).

For detecting the soluble protein, liquid nitrogen milled leaves (0.2 g) were extracted with precooled phosphate buffer (PH = 7.8) to achieve supernatant. The centrifugal condition was set as 12000 × g, 4°C for 20 min. 20 μl supernatant mixed with coomassie brilliant blue (G-250) dye solution for 2 min, the absorption at 595 nm was used to estimate the soluble protein content (Li et al, 2021). The protein standard was the bovine serum albumin.

Enzyme activity analysis

SOD (EC:1.15.1.1), POD (EC:1.11.1.7) enzyme activities analysis was carried out as previously reported with few modifications (Hu T et al, 2011). The crude enzyme was extracted by liquid nitrogen grinding and centrifugation from 0.2 g leaves or roots. A 3 ml reaction mixture for SOD was composed of 50 mM phosphate buffer (pH = 7.8), 195 mM Met, 20 mM riboflavin, 100 μM EDTA-Na_2, 750 μM NBT and 0.2 mL crude enzyme. The reaction system was illuminated for 30 min at 72 μmol m_{-2} s_{-1}, the absorbance at 560 nm was recorded. The reaction mixture for POD included 20 mM guaiacol, 100 mM PBS (pH = 6.0), 40 mM H_2O_2 and 0.2 mL enzyme extract. Mean change in absorbance per minute at 470 nm was measured.

The RuBPase (Ribulose-1,5-bisphosphate carboxylase, EC:4.1.1.39) and PEPCase (Phosphoenolpyruvate carboxylase, EC:4.1.1.31)enzyme activities were determined as described by Ding L et al, (2005). The

crude enzyme was prepared by extraction buffer containing 0.1 M Tricine-HCl (pH = 8.4), 10 mM MgCl2, 1 mM EDTA, 7 mM β-mercaptoethanol, 5% glycerol (v/v) and 1% polyvinylpyrrolidone (PVP) at 15000 × g, 4°C for 10 min.

The 2.5 ml reaction mixture for RuBPase activity examination contained 50 mM Tricine-HCl (pH = 8.0), 15 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 0.2 mM NADH, 5 mM ATP, 1mM EDTA, 10 mM $NaHCO_3$, phosphoglyceric phosphokinase (15U), glyceraldehyde-phosphate dehydrogenase (5U), 10 mM KCl and 0.1 ml crude enzyme. Incubating at 25^oC for 10 min with 20 mM $MgCl_2$ and 10 mM $NaHCO_3$ was essential for the activation of RuBPase. The consumption rate of NADH at 340 nm was defined as the activity of RuBPase once the reaction was triggered by 0.5 mmol RuBP.

The 2.5 ml reaction system for PEPCase activity consisted of 100 mmol Tris-HCL (pH = 9.2), 10 mmol $MgCl_2$, 10 mmol $NaHCO_3$, 0.16 mmol NADH, 0.5 mmol phosphoenolpyruvate (PEP), malate dehydrogenase (15U) and 0.5 ml crude enzyme. After constant water bath at 28^oC for 10 min, the reaction was initiated by PEP. The activity of PEPCase was estimated by the decrease rate of NADH at 340 nm.

RNA isolation and quantitative real-time PCR

mRNA was extracted according to the instructions of plant total RNA purification kit (Gmbiolab. Co., Ltd, Taiwan) and reversely transcribed to cDNA with Hifair™ II 1st Strand cDNA Synthesis Kit (including genome DNA removal procedure) (YEASEN, Shanghai, China). The transcriptome sequencing data provided the source of the gene sequences in this study and the primer sequences designed by oligo7 software were listed in Supplementary Table.

2 μl of cDNA template along with 10 μl SYBR Green master mix with low Rox (Yeasen, China), 0.5 μl forward primers, 0.5 μl reverse primers and 7 μl nuclease-free water mixed into the 20 μl reaction system. Then the qRT-PCR was operated by ABI Quantstudio 6 Flex real-time PCR system (Applied Biosystems, FosterCity, CA) with melting curves inspection at end of each reaction. *ACTIN* gene was used as a reference, each reaction had three duplications. The primer sequence is listed in Additional file 1.

Statistical Analysis

Above assays were performed with at least three independent replicates. The significance of differences was determined by ANOVA with Student-Newman-Keuls ($P < 0.05$) and marked by letters (a, b, c, d).

Declarations

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Availability of data and materials

Data sharing is not applicable to this article as all new created data is already contained within this article. RNA sequences of genes involving in manuscript are retrieved from the National Center forBiotechnology Information (NCBI) Sequence Read Archive (SRA) database (accession number: PRJNA645038) and listed in Additional file 2.

Authors' contributions

Guangyang Wang: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original Draft, Visualization; **Jinyan Mao**: Software, Investigation, Data Curation; **Mingxia Ji**: Investigation, Validation; **Wei Wang**: Methodology, Data Curation; **Jinmin Fu**: Resources, Writing - Review & Editing, Supervision, Project administration.

Ethics approval and consent to participate

Not applicable. The authors declared that experimental research works on the plants described in this paper comply with institutional, national and international guidelines. Use of plant material has been permitted.

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no conflicts of interest to this work.

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Figures

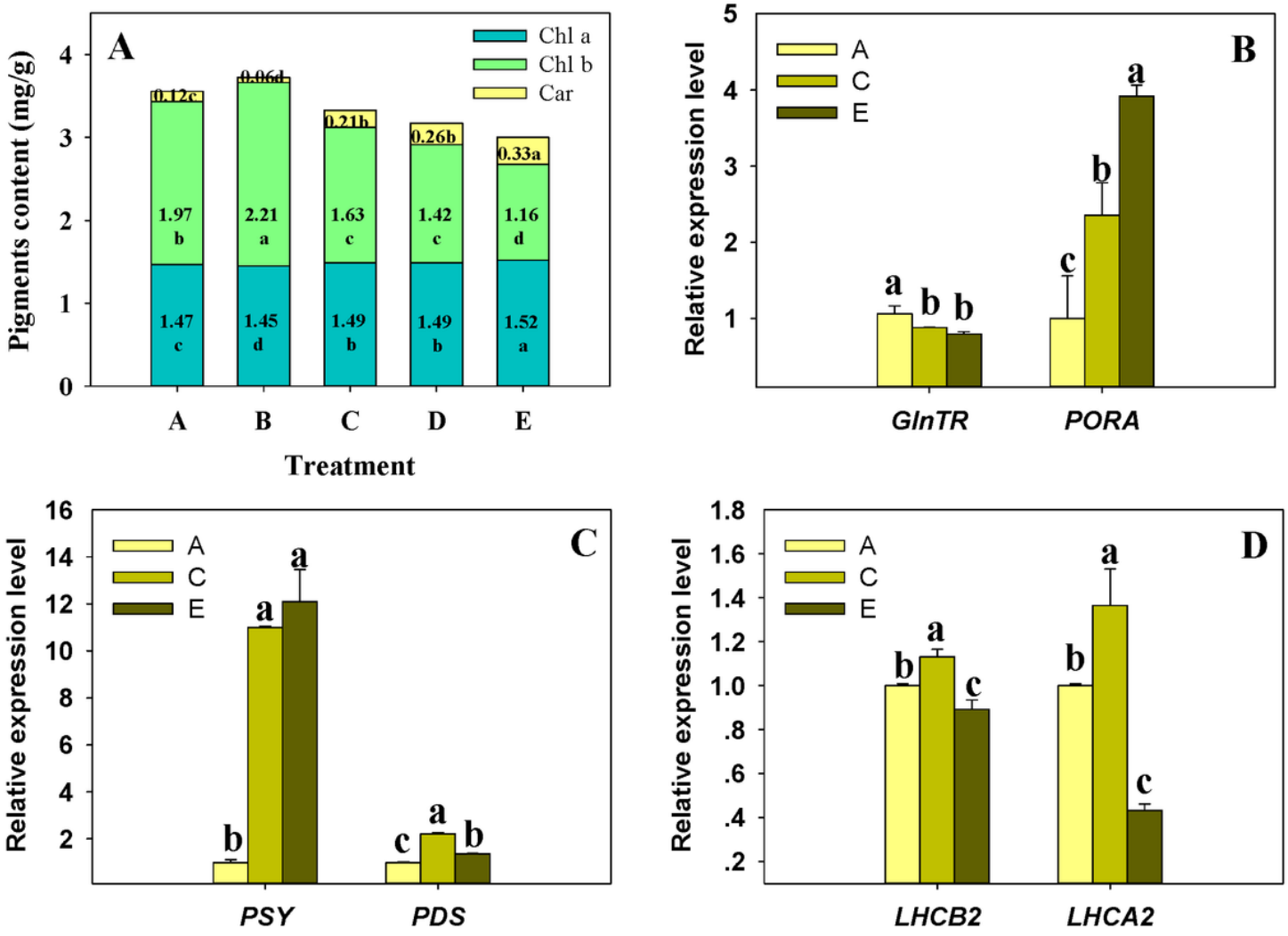

Figure 1

The adjustment of light harvesting capacity of Bermudagrass under shade.

The capital letters on the X axis represent the deepening shade (From A group to E group), represents the control (100%) and 50%, 25%, 12.5%, 6.25% shading treatment, respectively. The Student-Newman- Keuls multiple range tests were used to analyze statistical differences. (A) Photosynthetic pigment content (per gram of fresh weight), numbers and letters in boxes represent the mean values and statistical differences. The transcriptional levels of gene related to key enzymes in photosynthetic pigment synthesis pathway (B, C) and light harvesting complex (D) were detected. A group was defined as 1.

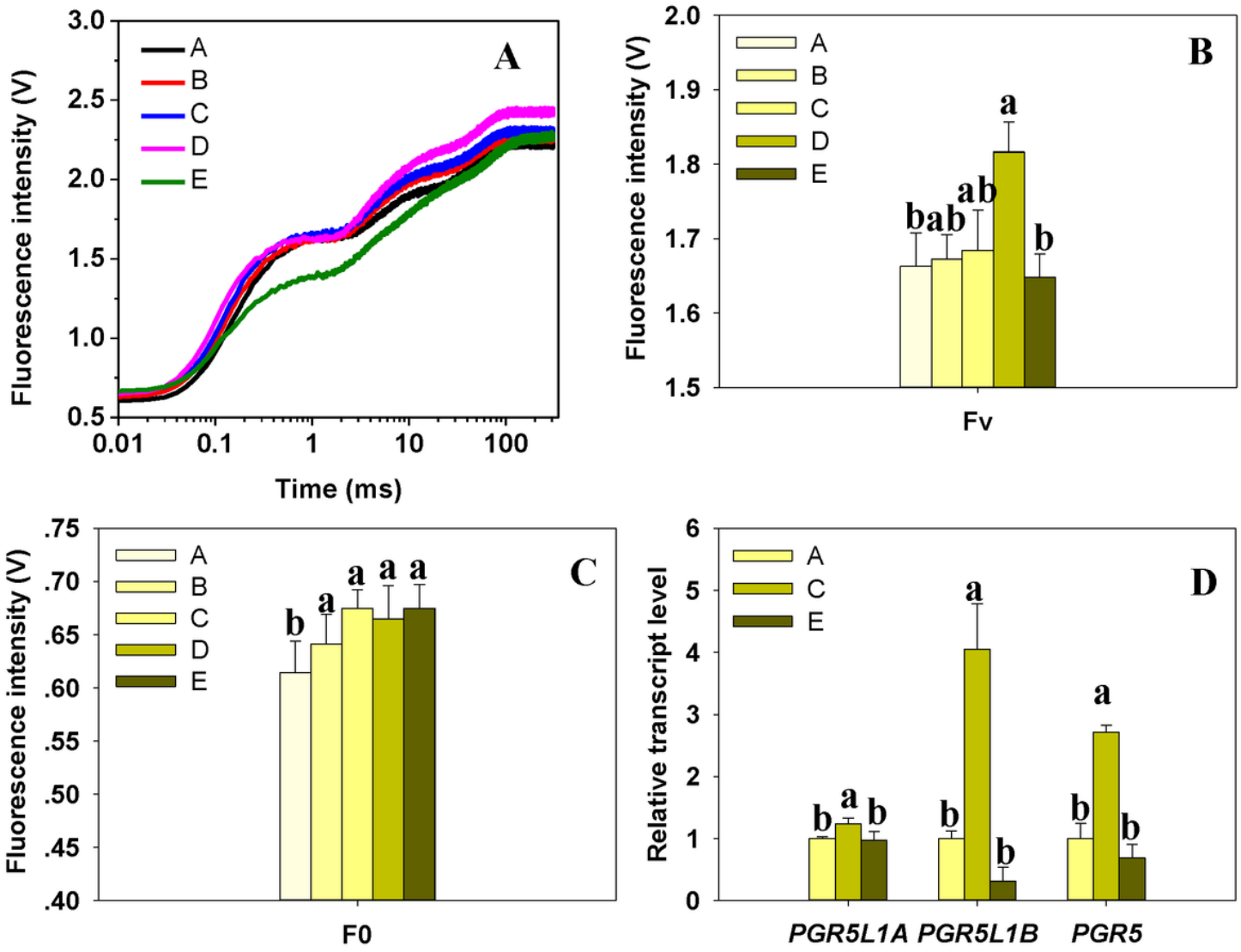

Figure 2

Effects of shading on fast chlorophyll fluorescence transients and cyclic electron transport of Bermudagrass. (A) Polyphasic rise of chlorophyll fluorescence; (B, C) The maximal variable fluorescence and initial fluorescence of PSII; (D) Gene transcriptional level of PGR5 complex subunit, A group was defined as 1.

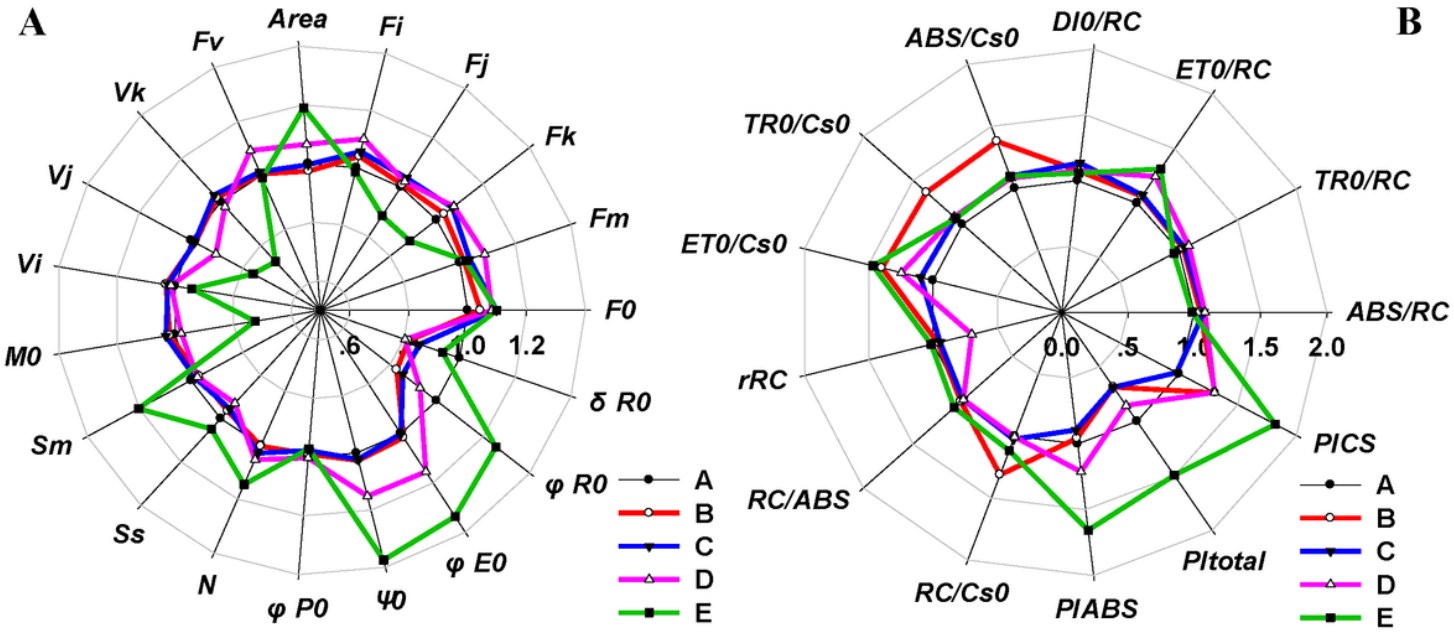

Figure 3

Radar plots of fluorescence transient parameters derived by JIP-test. Parameters information in detail was shown in Table 1.

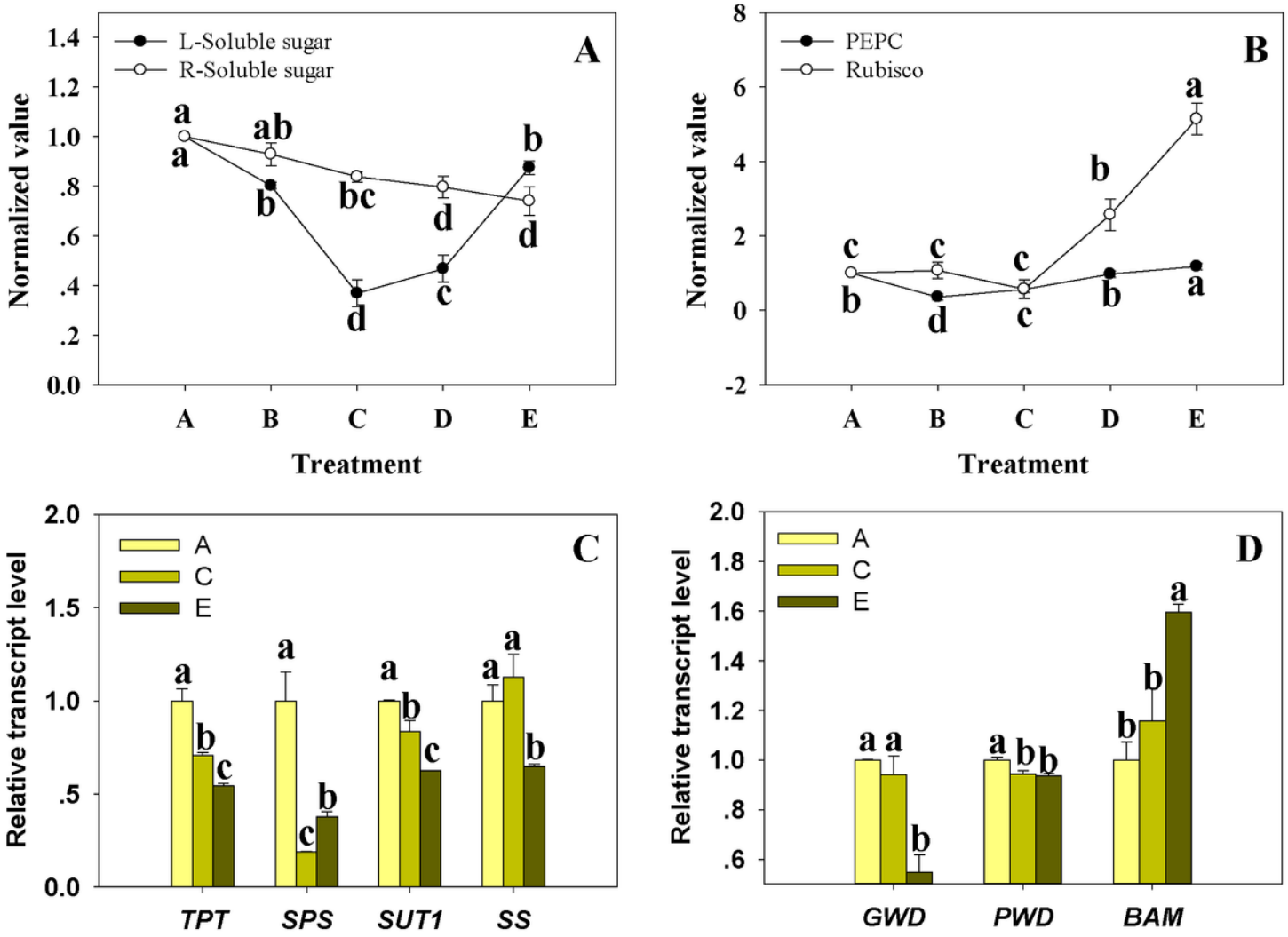

Figure 4

The carbon metabolism of Bermudagrass under shade.(A) Soluble sugar content in leaves (L) and roots (R); (B) Carbon assimilating enzyme activity; the transcriptional levels of gene related to sucrose metabolism and transport(C) and starch decomposition (D). A group was defined as 1.

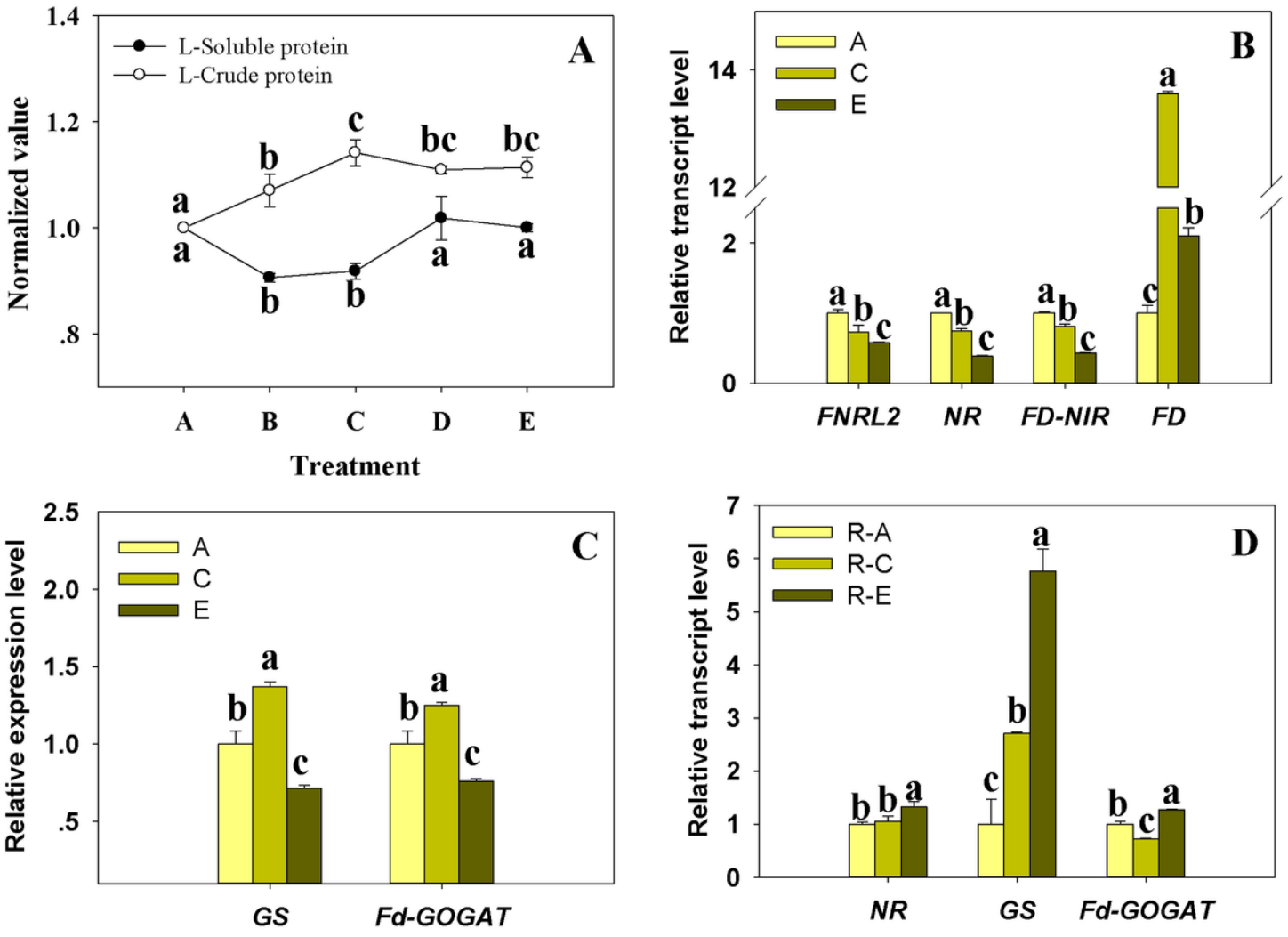

Figure 5

The nitrogen metabolisms in Bermudagrass under shade. (A) Soluble protein and crude protein content in leaf; (B, C, D) Transcriptional levels of genes related to nitrate reduction and ammonia assimilation. A group was defined as 1. In the legend, "A" and "R-A" represent leaves and roots of group A.

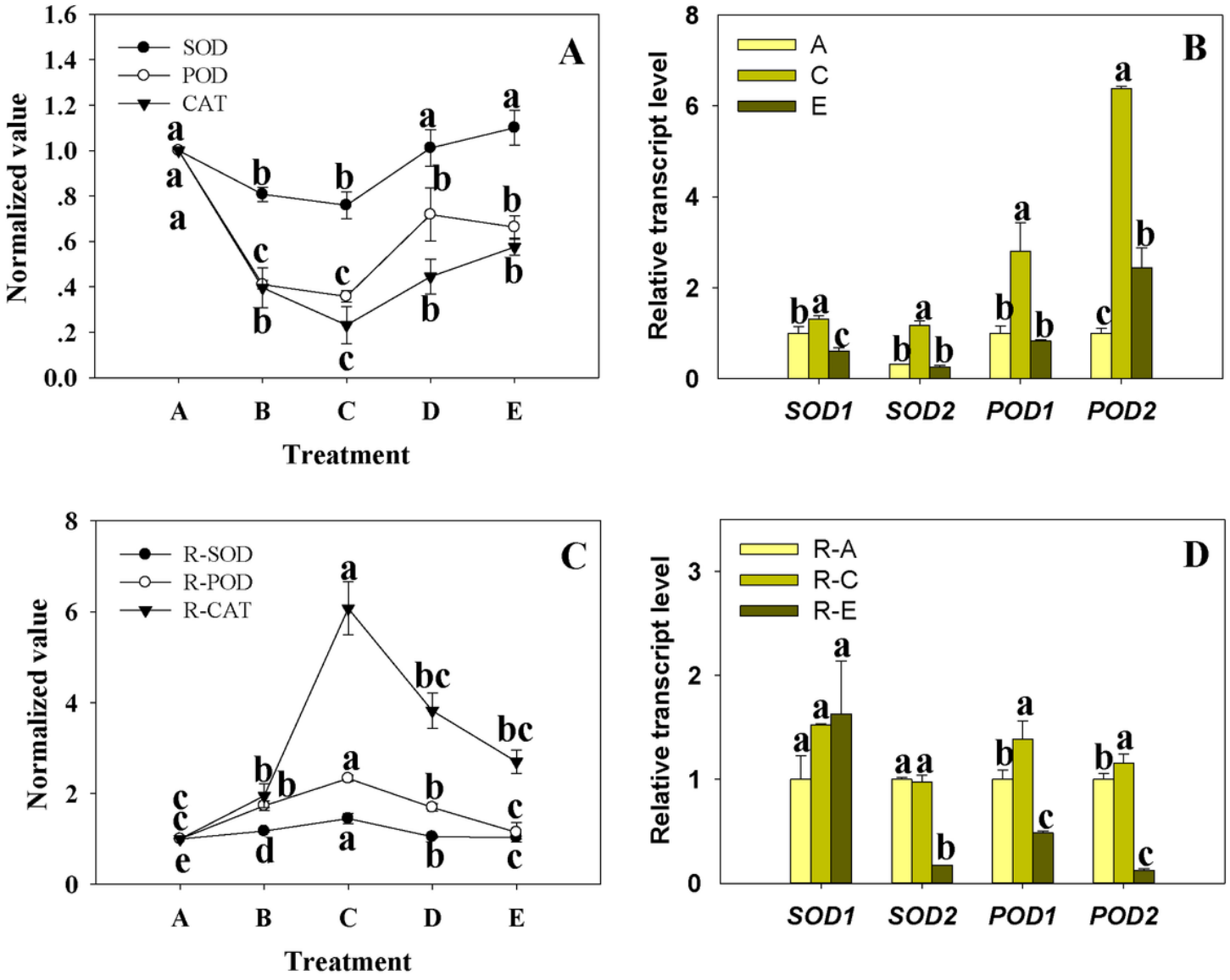

Figure 6

Antioxidant enzyme activities (A, C) and related gene expression (B, D) in roots and leaves under shading. In the legend, "R" represent roots. A group was defined as 1.

Supplementary Files

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- Additionalfile1.docx
- Additionalfile2.docx