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BRIEF COMMUNICATION

Expression of genes encoding key components of chlororespiration and cyclic electron transfer in soybean under different light qualities

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Abstract

Our present work showed that the expression of genes encoding PTOX (terminal oxidase of chlororespiration) and PGR5 (one essential component of cyclic electron transfer) were stimulated by red and blue light, but the stimulation under red light was soon reversed by subsequent far-red light. The expression levels of *PTOX* and *PGR5* under simulated light quality conditions in line with maize–soybean relay strip intercropping (SRI) were obviously lower than those under simulated soybean monocropping (SM), since the lower red:far-red ratio under SRI. Measurements on photosynthetic and chlorophyll fluorescence parameters suggested a decline of assimilatory power supply and a lower nonphotochemical quenching under SRI as compared to SM. In this case, weaker PGR-dependent cyclic electron transfer and chlororespiration under SRI, suggested by lower expression levels of *PGR5* and *PTOX*, could be considered as means of reducing excitation energy dissipation to allocate more power toward CO_2 assimilation.

Additional key words: chlorophyll fluorescence; NAD(P)H dehydrogenase; photosynthesis; shade.

Intercropping, a land-use strategy of cultivating two or more species simultaneously in the same piece of cropland, is a worldwide used agricultural practice (Lithourgidis *et al.* 2011). As a highly efficient utilization of resources, cereal–legume intercropping is recognized as a promising cropping system in many countries. In recent years, maize–soybean relay strip intercropping (SRI) has been developed rapidly in southwestern China, and recognized as a paradigm of sustainable agriculture, with the aim of enhancing food security (Yang *et al.* 2014). In this system, soybean is sown between rows of maize plants about two months before maize maturity. However, canopy of tall maize plants causes shading to growing soybean plants, and consequently also the restriction of crop productivity (Yang *et al.* 2014). Therefore, understanding to the response of relay-intercropped soybean to maize shading is necessary for further improvement of this system.

Plants have developed a complex set of mechanisms to adapt to shade, among which response of photosynthesis processes is regarded as a strategy of plants to cope with light energy-limited environment. Previous studies showed that relay-intercropped soybean always shows reduced photosynthetic capacity and some shade tolerance traits, such as increased specific leaf area and chlorophyll (Chl) content, under maize shading (Su *et al.* 2014a, Gong *et al.* 2015). But the regulation mechanisms hidden behind photosynthetic performance remain to be clarified. It is well-known that shade does not only lead to decreased light intensity, but also induces changes of light quality. Yang *et al.* (2014) found that the light intensity and red:far-red (R/FR) light ratio of soybean canopy in maize–soybean relay strip intercropping decreased significantly as compared to soybean monocropping, and the responses of relay intercropped soybean to maize shading were caused by the summed effects of both reduced light intensity and the R/FR ratio. But regrettably, for the effects of shade on

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Abbreviations: CET – cyclic electron transfer; C_i – intercellular CO_2 concentration; Chl – chlorophyll; ETR – electron transport rate; F_v/F_m – maximum photochemical efficiency of PSII photochemistry; g_s – stomatal conductance; LED – light-emitting diode; NDH – NAD(P)H dehydrogenase; NPQ – nonphotochemical quenching; PGR – proton gradient regulation; PQ – plastoquinone; PTOX – plastid terminal oxidase; q_P – photochemical quenching coefficient; R/FR – red:far-red; RbcS – Rubisco small unit; SM – simulated monocropping; SRI – simulated relay strip intercropping.

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photosynthetic performance, most previous studies were mainly focused on reduced light intensity. Nevertheless, there are still reports indicated that different light qualities have effects on photosynthetic characteristics of plants. For example, red light has been suggested to play a role in the development of photosynthetic apparatus and regulation of electron transport (Sæbø *et al.* 1995, Yu *et al.* 2016). Blue light was found to be important for Chl biosynthesis, chloroplast development, and stomata movement (Hogewoning *et al.* 2010, Su *et al.* 2014b). But how the changes of light quality influence photosynthesis processes is poorly understood.

In the present work, we studied the expression of genes encoding key components of chlororespiration and cyclic electron transfer (CET) around PSI in soybean under different light qualities, including simulated conditions in line with maize–soybean relay strip intercropping (SRI) and soybean monocropping (SM), respectively. Chlororespiration refers to a respiratory electron transport chain, which interacts with photosynthetic electron transport chain in the thylakoid membrane of chloroplasts, with a terminal oxidase called PTOX (plastid terminal oxidase) (Peltier and Cournac 2002). The function of chlororespiration has been indicated to have a close relationship with CET (Savitch *et al.* 2010, Ivanov *et al.* 2012). Evidence proved the existence of two CET pathways, which are dependent on NAD(P)H dehydrogenase (NDH) complex and proton gradient regulation 5 (PGR5), respectively, in higher plants (Suorsa 2015). Cooperation of CET with chlororespiration has a potential ability to fine-tune the redox state of electron carriers in thylakoid membrane, and could be considered as a strategy of plants to cope with different light conditions which may cause effects on photosynthesis (Rumeau *et al.* 2007, Sun and Wen 2011).

Our present work also studied some related photosynthetic and Chl fluorescence parameters under different light qualities in order to elucidate the photosynthetic characteristics of soybean under the light quality condition in maize–soybean relay strip intercropping system.

Seeds of soybean [*Glycine max* (L.) Merr.] cv. Gongxuan 1, a cultivar usually planted in maize–soybean relay strip intercropping system, were germinated and grown in a greenhouse [temperature: 25°C day/20°C night; photoperiod: 14 h light/10 h dark; relative humidity: 75%; irradiance: 550 μmol(photon) m^{-2} s^{-1}]. One-month-old soybean plants were adapted to total darkness before treated, and then exposed to different light qualities from three kinds of light-emitting diode (LED) lamps (blue, red, and far-red light) in a growth chamber. For single light-quality treatments, plants were exposed to red, far-red, and blue light, respectively. Light quality conditions of SRI (red:far-red:blue = 1:0.88:1) and SM (red:far-red:blue = 1:0.68:1) were supplied according to Liu *et al.* (2011), using different combination of the three light qualities. The light intensities of SM and SRI treatments were kept at the same level, with purpose of studying the effects of only light quality. For experiments, the latest fully expanded leaves were used.

Total RNA was extracted from liquid nitrogen-powdered leaf tissue according to Sun *et al.* (2014). Reverse transcription PCR was carried out in a *MyCycler* gradient PCR amplifier (*Bio-Rad*, Richmond, CA, USA) using first-strand cDNA, to detect the transcripts of *PTOX*, *PGR5*, *NDH-H* (H subunit of NDH complex) and *RbcS* (Rubisco small unit). Gene-specific primers used for PCR detection were as described by Sun *et al.* (2014) and Liu (2016). The levels of transcripts were relative-quantified with software *Image Lab* (*version 4.1*).

Content of Chl was determined according to Lichtenthaler and Buschmann (2001). Stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were measured by an open system under room temperature and CO_2 partial pressure, using two cross-calibrated infrared gas analyzers with a 2 cm^2 fluorometer chamber (*LI-6400*, *Li-COR Inc.*, USA). Chl fluorescence was measured by a Chl fluorescence imaging system (*CF Imager*, *Technologica*, UK), following the manufacturer's instructions. Maximum photochemical efficiency of PSII photochemistry (F_v/F_m), photochemical quenching coefficient (q_P), electron transport rate (ETR), and nonphotochemical quenching (NPQ) were calculated by software *CF Imager* (*version 2.2*) according to Baker and Oxborough (2004).

All results were means of five independent experiments. Data were analyzed using *SPSS* (*version 13*) for *Windows* software (*SPSS*, Chicago, USA), and statistically evaluated using the standard deviation and *t*-test methods. The difference was considered to be statistically significant when $P<0.05$.

Gene transcript detection showed that the expression of both *PTOX* and *PGR5* were induced in red light-treated soybean plants (Fig. 1*A*). Expression of these two genes increased to relatively high levels within a duration of about 90 to 120 min, then remained stable (Fig. 1*A*,*E*). This result suggested that chlororespiration and CET of soybean might be enhanced under red light. However, the expression of *NDH-H* showed no response to red light (Fig. 1*A*,*E*), indicating the possible enhancement of CET relied only on PGR-dependent pathway, but not the activity of NDH complex. The expression of *RbcS* also remained at the same level after red-light treatment (Fig. 1*A*,*E*), indicating that a content of Rubisco, the key enzyme for CO_2 assimilation, remained unchanged. When soybean plants were transferred to far-red light after 120 min of red-light treatment, the expression levels of both *PTOX* and *PGR5* decreased obviously, while the expression of *NDH-H* and *RbcS* showed no response (Fig. 1*B*,*E*). Effects of blue light were also studied. Results showed that the expression levels of *NDH-H* and *RbcS* still remained unchanged, while levels of *PTOX* and *PGR5* increased obviously, just as those under red light (Fig. 1*C*,*E*). Therefore, a role of light receptors, such as phytochrome for R/FR light signal and cryptochrome for blue light signal, could be proposed in the regulation of

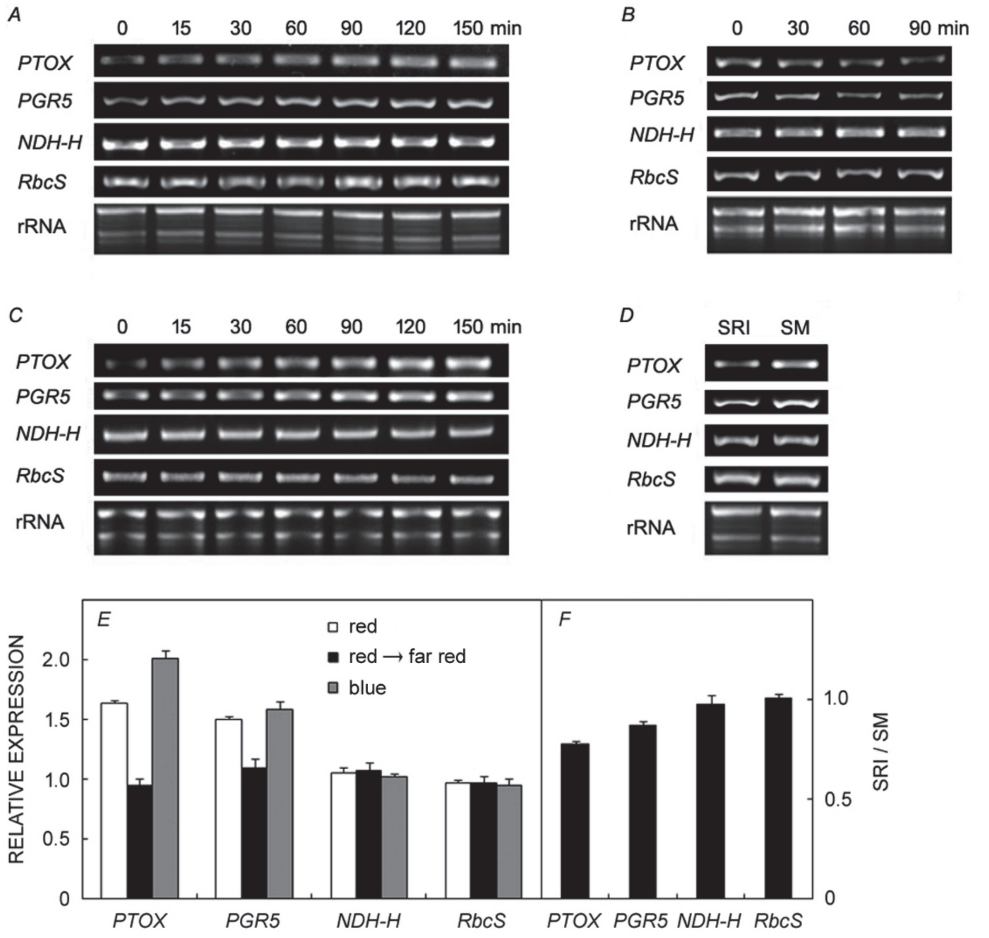

Fig. 1. Expression analysis on *PTOX*, *PGR5*, *NDH-H*, and *RbcS* genes in soybean under different light qualities. Transcript detection under red light for 150 min (*A*), far-red light for 90 min after red light for 120 min (*B*), blue light for 150 min (*C*), and simulated light qualities in line with soybean monocropping (SM) or maize-soybean relay strip intercropping (SRI) for 120 min (*D*), respectively. rRNA is shown as a loading control and indicator of RNA intactness. Relative expression levels are shown in (*E*) for 120 min of red light, 90 min of far-red light after 120 min of red light, and 120 min of blue light. Ratio of expression levels under SRI and SM is shown in (*F*). The level at 0 h is defined as 1.0. Bars represent the standard deviations of five independent replicates.

Table 1. Photosynthetic and fluorescence parameters of soybean under simulated light qualities in line with soybean monocropping (SM) and maize-soybean relay strip intercropping (SRI) for 120 min. Values represent means ± SD, *different letters* indicate statistical difference at $P<0.05$.

Parameter	Treatment SM	SRI
Chl (*a*+*b*) [mg g^{-1}]	1.669 ± 0.065^{b}	1.912 ± 0.069^{a}
Chl *a*/*b*	3.359 ± 0.276^{a}	2.838 ± 0.300^{b}
g_s [mol(H$_2$O) m^{-2} s^{-1}]	0.064 ± 0.008^{a}	0.055 ± 0.012^{b}
C_i [μmol(CO$_2$) mol^{-1}]	236.73 ± 13.75^{b}	251.50 ± 7.11^{a}
F_v/F_m	0.831 ± 0.010^{a}	0.810 ± 0.009^{b}
q_P	0.829 ± 0.017^{a}	0.810 ± 0.019^{a}
ETR [μmol m^{-2} s^{-1}]	12.50 ± 4.89^{a}	8.14 ± 3.74^{b}
NPQ	0.277 ± 0.016^{a}	0.195 ± 0.021^{b}

PTOX and *PGR5* expression. Consequently, changes in light quality had potential effects on chlororespiration and PGR-dependent CET of soybean.

Further detection and analysis found that the expression levels of *PTOX* and *PGR5* under SRI were obviously lower than those under SM (Fig. 1*D*,*F*). This difference should be caused by the far-red light reversion on gene expression under a relatively lower R/FR ratio of SRI. Expression of *NDH-H* and *RbcS* still remained at the same levels under both simulated light conditions (Fig. 1*D*,*F*). These results suggested that chlororespiration and PGR-dependent CET were probably restricted under SRI as compared to SM.

Content of total Chl (*a*+*b*) in soybean leaves under SRI was obviously higher than that under SM, with a significant decline of Chl *a*/*b* (Table 1). These results indicated that SRI caused an apparent shade-tolerance trait in soybean plants, and the higher Chl content under maize shading under field conditions should occur due to the changes in light quality (R/FR ratio), since decrease of light intensity had no clear effect on it (Liu 2016). Higher

Chl content also implied an enhanced light-harvesting ability of leaves under SRI, which was benefit for the photosynthesis of soybean plants under light energy-limited environment.

Gas-exchange measurements found that g_s of soybean plants under SRI was lower than that under SM. Simultaneously, C_i under SRI was shown to be obviously higher than that under SM (Table 1). These results indicated a decline of CO_2 assimilation in soybean plants under SRI as compared to SM. Expression detection suggested that the content of Rubisco kept at a same level in plants under SRI and SM (Fig. 1*D*), implying similar ability of CO_2 carboxylation under both light quality conditions, although the activity of Rubisco was not determined in the present study. Therefore, restricted supply of assimilatory power should be regarded as an important reason, at least partly, for the decline of CO_2 assimilation under SRI. Determination of Chl fluorescence showed a significantly lower level of ETR in soybean under SRI than that under SM (Table 1), further conforming the supply of assimilatory powers was lowered under SRI. Moreover, F_v/F_m was detected to decrease obviously under SRI, and q_P displayed no clear difference between SRI and SM (Table 1). These results indicated that the restriction on assimilatory power supply under SRI was related to the decreased ability of light energy conversion, although the light-harvesting ability might be enhanced by the increased Chl content.

In addition, it is worth to notice that the level of NPQ under SRI was significantly lower than that under SM (Table 1), suggesting a decreased ability of dissipating excitation energy under SRI. This implied that energy allocation in SRI-treated soybean was more inclined to CO_2 assimilation, under the background of restriction on assimilatory power supply. Previous studies found that the activation of NPQ is dependent, at least partly, on the building-up of proton gradient across thylakoid membranes (∆pH) by CET (Shikanai 2014, Suorsa 2015). Studies also showed that function of CET have a close relationship with chlororespiration. CET pathways cycle electrons from PSI back to the plastoquinone (PQ) pool, and then electrons can be transferred to O_2 by PTOX (Rumeau 2007, Shikanai 2014). So, cooperation of CET with PTOX has a potential ability to fine-tune the redox state of electron carriers in thylakoid membrane (Savitch *et al*. 2010, Ivanov *et al*. 2012). In our present study, the much lower expression level of *PGR5* and *PTOX* under SRI suggested that the decrease of NPQ was caused by the decline of PGR-dependent CET, and also related to a weaker chlororespiration. Therefore, the regulation of gene expression of key components in chlororespiration and CET could be considered as a strategy of soybean to acclimate to light energy-restricted environment under shade, in order to allocate more power towards photosynthate production.

In conclusion, our present study suggested that changes in light quality have potential effects on chlororespiration and CET of soybean by regulating the gene expression of key components, although the expression level of genes may not reflect their activity. The expression levels of *PGR5* and *PTOX* under SRI were obviously lower than those under SM. These responses were suggested to be means of reducing excitation energy dissipation, and could be considered as a strategy of intercropped soybean to use energy more efficiently when supply of assimilatory powers was restricted under shade.

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ANNOUNCEMENT

The 8th International Conference on *Photosynthesis and Hydrogen Energy Research for Sustainability-2017*, October 30 – November 3, 2017, Hyderabad, India: An Invitation

On behalf of the Organizing Committee, all of us have the pleasure to cordially invite you to participate in the 8th International Conference on *Photosynthesis and Hydrogen Energy Research for Sustainability-2017*, being organized in honor of ***Agepati S. Raghavendra*** (of University of Hyderabad, India; **http://www.uohyd.ac.in/images/academics/school_study/lifescience/plantsciences/asr_170117.pdf**, ***William A. Cramer*** (of Purdue University, USA; **https://www.bio.purdue.edu/lab/cramer/**), and ***Govindjee*** (of University of Illinois at Urbana-Champaign; **http://www.life.illinois.edu/govindjee/**)

The meeting will be held from October 30 to November 03, 2017 at the School of Life Sciences, University of Hyderabad, Hyderabad, India. We are proud to note that the University of Hyderabad (**http://www.uohyd.ac.in/**) is one of the top Universities in India. The University is largely devoted to postgraduate studies and is widely known for its excellence in research and its distinguished faculty. Founded in 1974 by an act of parliament, the Hyderabad Central University, also known as University of Hyderabad, has emerged as a leading center of teaching and research. Its large campus is located near *Gachibowli*, an Information Technology (IT) hub of Hyderabad city; it has immense bio-diversity across its extensive campus of over 2,000 acres of green land. It is home for over 75 varieties of birds, not to speak of the lakes and rock formations, which give the campus a natural beauty. Please come and enjoy not only the meeting, but the city and the country.

This Meeting will be a great occasion for discussions of previous, present, and future research on "***Photosynthesis and Hydrogen Energy, from molecular to global*"**. It will provide an exciting scientific program, which will cover all aspects from the basics to their applications. It will be a great forum for students, postdoctoral fellows and established scientists from different countries to exchange ideas, and to broaden their knowledge. It will be an excellent occasion to meet researchers from around the world, widen professional contact and create new opportunities, including establishing new collaborations.

Lectures and posters presented at the Conference will be selected, peer-reviewed and published in special issues of Photosynthesis Research (Springer) and International Journal of Hydrogen Energy (Elsevier) in the form of regular articles or reviews. For further information, details and conference updates, **please visit the conference website: https://prs.science/**, which will contain all information on the topics, program, deadlines, accommodations, and travel information. The website will be regularly updated. We already have received confirmation from many international scientists from around the World.

Your participation is important for the success of this meeting, and will be very much appreciated. Looking forward to seeing you in Hyderabad soon. Please feel free to write to any of us listed below (*corresponding author: Rajagopal Subramanyam).

Sincerely yours,

- **James Barber (Chair**; London, **U.K**.; e-mail: j.barber@imperial.ac.uk)
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- ***Rajagopal Subramanyam** (**Co-Chair and Organizing Secretary**; Hyderabad, **India**; email: prsuoh2017@gmail.com; psrajagopal@yahoo.com)
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- **Julian J. Eaton-Rye (Co-chair & Secretary International Society for Photosynthesis Research**; Dunedin, **New Zealand**; e-mail: julian.eaton-rye@otago.ac.nz)
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