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Light quality modifies the expression of photosynthetic genes in maize seedlings

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Abstract

Although maize (*Zea mays* L.) plants utilize light efficiently, the expression of high light-efficient genes and stomatal factors is regulated by light conditions and affects photosynthesis of plants. In this study, we investigated the effects of different light qualities on the expression of the photosynthetic genes, such as *pep1*, *pdk1*, *ZmSTOMAGEN*, and *psad1*, and on stomatal function in maize seedlings. For both maize genotypes, Zhengdan 958 and Xianyu 335, light with wavelengths shorter than 490 nm enhanced the expression of *pdk1* and *ZmSTOMAGEN*, whereas the expression of *pdk1* positively correlated with *ZmSTOMAGEN*. Light with wavelengths longer than 630 nm or shorter than 490 nm (band pass filter) increased the expression of *pep1* and *psad1*. Although the expression of four genes in Zhengdan 958 was significantly higher than that of Xianyu 335, changes in the expression of *ZmSTOMAGEN*, *pdk1*, or *pep1* exerted no significant influence on stomatal function and photosynthetic rate. Our results suggest that light with wavelengths shorter than 490 nm promoted the expression of stomatal proteins and *pdk1*, facilitated the absorption of inorganic elements, and contributed to stomatal function in photosynthesis. Meanwhile, light with wavelengths longer than 630 nm inhibited the expression of *pep1* and *pdk1*. Light with wavelengths longer than 630 nm or shorter than 490 nm promoted the expression of *pep1*, *pdk1*, and *psad1*.

Additional key words: gas exchange; *pdk1*; *pep1*; phospho*enol*pyruvate carboxylase; *psad1*; stomata.

Introduction

Light is an important environmental factor that affects plant growth and development. Light is converted into chemical energy through photosynthesis. Light is also involved in several other biochemical processes, such as specific, light-induced seed germination (Shinomura *et al.* 1994), cotyledons opening (Casal and Boccalandro 1995), phototropism (Whippo and Hangarter 2003), root development (Galen *et al.* 2007), flavonoid compound synthesis (Jackson and Jenkins. 1995), and anthocyanin biosynthesis (Duke *et al.* 1976). It regulates xanthochrosim (Shinomura *et al.* 1994), stomatal behavior (Kinoshita *et al.* 2001), plant photoperiod, and flowering time (Ueoka-Nakanishi *et al.* 2012) and suppresses seedling hypocotyl elongation (Ahmad and Cashmore 1993), and plant gravitropism (Robson and Smith 1996). However, light can also be a stress factor. Light intensity and quality can decrease the crop photosynthetic capacity and destroy structures of the photosynthetic apparatus (Lichtenthaler *et al.* 1981, Ivanova *et al.* 2008, Huang *et al.* 2011, Yamazaki and Shinomiya 2013). In order to better adapt to the change of light, plant can adjust its structure of the photosynthetic apparatus to enhance the absorption and utilization

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Abbreviations: BP – band pass; Chl – chlorophyll; CL – transparent glass; *E* – evapotranspiration; Fd – ferredoxin; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; g_s – stomatal conductance; LP – long-band pass; PEPC – phospho*enol*pyruvate carboxylase; phyB – phytochrome B; P_N – net photosynthetic rate; PPDK – pyruvate, orthophosphate dikinase; SP – short-band pass; VPD – vapor pressure deficit; WUE – water-use efficiency.
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of light (Lichtenthaler *et al.* 1981, Wada *et al.* 2003, Niinemets 2010).

Almost all terrestrial plants possess stomata on their epidemis; the stomata have developed various features throughout plant evolution and differentiation (Ruszala *et al.* 2011). Plants detect changes in gas concentration, humidity, temperature, light environment, and other external signals and then adjust the movement, size, and number of stomata accordingly in order to optimize the capacity of gas exchange and to adapt to environmental changes (Nadeau and Sack 2002). Stomatal movement and density determine the capacity and efficiency of plant photosynthesis. The shape, size, distribution, and number of stomata significantly affect plant physiological processes, such as photosynthesis and transpiration. Meanwhile, stomatal density is controlled by genetic and environmental factors (*e.g.*, temperature, CO_2 concentration, and water conditions) and cultivation methods; different light qualities exert different effects on stomatal functions (Zeiger 1983, Xie *et al.* 2006, Lampard *et al.* 2009, Okamoto *et al.* 2009, Casson and Hetherington 2010, Liu *et al.* 2011).

Light facilitates plant photosynthesis. Light intensity can modify the stomatal index (Schoch *et al.* 1980, Thomas *et al.* 2004, Jiang *et al.* 2011) and light quality can influence the stomatal index and density (Shimazaki *et al.* 2007, Boccalandro *et al.* 2009). Stomagen is the only secreted oligopeptide signaling protein which regulates positively the generation of stomata. High stomagen expression increases leaf stomatal density and enables plants to augment the photosynthetic rate substantially (Tanaka 2013). Several studies have analyzed the implications of stomatal development and the underlying environmental regulatory mechanism in plant evolution as well as in application of modern agricultural and environmental indicators. However, the mechanism by which light factors influence stomatal development remains poorly understood.

The *pep1* gene encodes phospho*enol*pyruvate carboxylase (PEPC) which catalyzes the irreversible carboxylation of phospho*enol*pyruvate (PEP) to yield oxaloacetate (Chollet *et al*. 1996), whereas *pdk1* encodes pyruvate, orthophosphate dikinase (PPDK), which plays a controlling role in the PEP-regeneration phase of the C_4 photosynthetic pathway (Chastain *et al*. 2011, Wang *et al*. 2012). *ZmSTOMAGEN* is a positive regulatory gene in stomatal development that has been recently discovered in the stomagen of maize, while *psad1* is the key gene of PsaD that participates in the linear electron transport of the PSI reaction center (Lin *et al*. 2009). In the present study, we chose these four genes from maize to analyze their expression and relationship with photosynthesis under different light conditions.

Materials and methods

Site description and soil sampling: The experimental site was located at the Center of Excellence for Research in Optoelectronic Agriculture (26°09′N, 119°23′E) of the College of Mechanical and Electronic Engineering, Fujian Agriculture and Forestry University, in Fuzhou, China. The maize cultivars ‘Xianyu 335’ (which is the cultivar with wider adaptability, female parent is PH6WC, male parent is PH4CV) and ‘Zhengdan 958’ (which is lodging- and drought-resistant cultivar, female parent is Zheng 58, male parent is Chang 7-2) were grown in pots (260 mm × 135 mm × 70 mm) irradiated by solar radiation under each of the four novel filters designed by our laboratory:

Filter	Description	Transmitted wavelengths	Transmittance
SP	Short-band pass filter; it can transmit solar radiation and block all other radiation of long wavelengths	Shorter than 490 nm	Higher than 90%
LP	Long-band pass filter; it can transmit the solar radiation and block all the other radiation of short wavelenghts	Longer than 630 nm	Higher than 90%
BP	Band-pass filter; it can transmit solar radiation and block the radiation outside the given range	Longer than 630 nm or shorter than 490 nm	Higher than 90%
CL	Glass control; it can not filter any light		

The maize was cultivated for 15 d, the fifth leaf was chosen for sampling and testing. The plants were cultivated under ambient humidity and temperature with eight replicates. The experiments were performed from July to August in 2014 and 2015. The basic soil nutrition without any other additional fertilizer is described below.

	N [mg kg^{-1}]	P [mg kg^{-1}]	K [mg kg^{-1}]
Ck soil	8,947.78 ± 31.07	2,828.00 ± 10.12	14,954.59 ± 28.87

Photosynthesis: The net photosynthetic rate (P_N), stomatal conductance (g_s), vapor pressure deficit (VPD),

evapotranspiration (*E*), and water-use efficiency (WUE) were measured using the *CIRAS-3* portable photosynthesis system (*PP Systems*, Amesbury, MA, USA) equipped with a LED leaf chamber in the middle of each leaf with five replicates. The CO_2 concentration in the leaf chamber was 450 μmol s^{-1}, and ambient temperature and humidity were set in the chamber. The data were read when the PPFD was 1,800 μmol m^{-2} s^{-1} at a clear day from 10:00 to 12:00 h.

RT-PCR: Total RNA was isolated using the *RNAqueous® Total RNA Isolation Kit AM1912* (*Life Technologies Corp*., Grand Island, New York, USA), and reverse transcription was performed using the *iScript cDNA Synthesis Kit* (*BIO-RAD*, USA). Quantitative PCR was performed with the *ABI Power SYBR Green PCR MasterMix* (*ABI*, USA) and detected with the *7900 HT* Sequence Detection System (*ABI*, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a gene reference in the quantitative analysis of maize genes. Primers were designed in accordance with the published sequences of the *ZmSTOMAGEN* (NM_001149748.1), *pdk1* (GU363532), *pep1* (FJ415327.1), and *psad1* (EU953246.1) genes. The genes and primers are described as follows:

Gene id	Primer sequence (5' to 3')
pep1 (FJ415327.1)	F 5' – ACCTGCTGGAGATGGTTTTC – 3'
	R 5' – GGTGATGTAGGGGTTGCG – 3'
pdk1 (GU363532)	F 5' – GTGGAGAACACGGTGGAGAG – 3'
	R 5' – CTGAAAGGGGAGCAAGAAACG – 3'
psad1 (EU953246.1)	F 5' – GGAGCAGGTGTTCGAGATG – 3'
	R 5' – TGGGGAAGACGCGGTAG – 3'
ZmSTOMAGEN (NM_001149748.1)	F 5' – TTCCTTCTCTCTTGCCTGCTT – 3'
	R 5' – AGACCCTTGATGACCTTCGG – 3'
Reference (GAPDH)	F 5' – TGGTACGACAACGAGTGGGG – 3'
	R 5' – GAGAAGCAGGGGGGAAAAAC – 3'

Physiological response, inorganic elements and stomata density: Chlorophyll (Chl) was extracted by acetone extraction and determined by *UV2600* spectrophotometer (*Shimadzu*, Japan) using the method described by Zhang (1992) based on the proposal by Arnon (1949). Inorganic elements Mg, K, Ca, N, and P were determined with a *HITACHI 180-70* flame atomic absorption spectrophotometer (*Hitachi*, Tokyo, Japan) using the method described by Pohl *et al*. (2012). The experiments were performed five times. The stomatal density was calculated for ten replicates by observing the middle part of the fifth leaf under a microscope at 100× and with 400× magnifications.

Statistical analysis: Analysis of variance (*ANOVA*) was used to analyze the significant differences between the measured data by comparing their means. The significance level was set as $\alpha = 0.05$. Multiple comparisons were used to determine the least significant difference at $\alpha = 0.05$. All of statistical results were caculated by *SPSS 17.0*.

Results

Effects of light filters on photosynthesis: To test the effects of four different illumination conditions caused by the three filters and the CL glass (the control), gas-exchange parameters, P_N, g_s, VPD, *E*, and WUE, were determined (Table 1). In Zhengdan 958, P_N, g_s, and *E* were 31.3, 56.4, and 43.2% lower, respectively, under the LP

Table 1 Photosynthesis parameters under different light conditions. Data are mean ± SD. *Different letters* in the table show significant differences determined by LSD test ($p<0.05$). P_N – net photosynthetic rate; g_s – stomatal conductance; VPD – vapor pressure deficit; *E* – evapotranspiration; WUE – water-use efficiency; CL – transparent glass; SP – short-band pass; LP – long-band pass; BP – band pass.

Treatment		P_N [μmol(CO_2) $m^{-2}s^{-1}$]	g_s [mol(H_2O) $m^{-2}s^{-1}$]	VPD [kPa]	*E* [mmol(H_2O) $m^{-2}s^{-1}$]	WUE [%]
Xianyu 335	CL glass	27.2 ± 1.9^{a}	134 ± 6^{a}	2.27 ± 0.14^{a}	2.73 ± 0.14^{a}	9.95 ± 0.31^{ab}
	SP filter	23.0 ± 4.8^{a}	123 ± 8^{a}	2.26 ± 0.26^{a}	2.43 ± 0.50^{a}	9.46 ± 0.40^{b}
	BP filter	26.8 ± 3.1^{a}	130 ± 9^{a}	2.15 ± 0.09^{a}	2.52 ± 0.20^{a}	10.59 ± 0.34^{a}
	LP filter	22.7 ± 3.2^{a}	120 ± 8^{a}	2.18 ± 0.37^{a}	2.18 ± 0.33^{b}	10.41 ± 0.07^{a}
Zhengdan 958	CL glass	28.7 ± 1.0^{a}	101 ± 5^{a}	2.45 ± 0.07^{b}	2.29 ± 0.11^{a}	12.54 ± 0.58^{c}
	SP filter	24.0 ± 1.0^{b}	81 ± 9^{b}	2.47 ± 0.20^{b}	1.86 ± 0.03^{b}	12.93 ± 0.64^{c}
	BP filter	21.2 ± 2.5^{bc}	58 ± 6^{c}	2.67 ± 0.19^{b}	1.47 ± 0.07^{c}	14.35 ± 0.28^{b}
	LP filter	19.7 ± 1.5^{c}	44 ± 7^{d}	3.10 ± 0.11^{a}	1.30 ± 0.17^{c}	15.64 ± 0.33^{a}

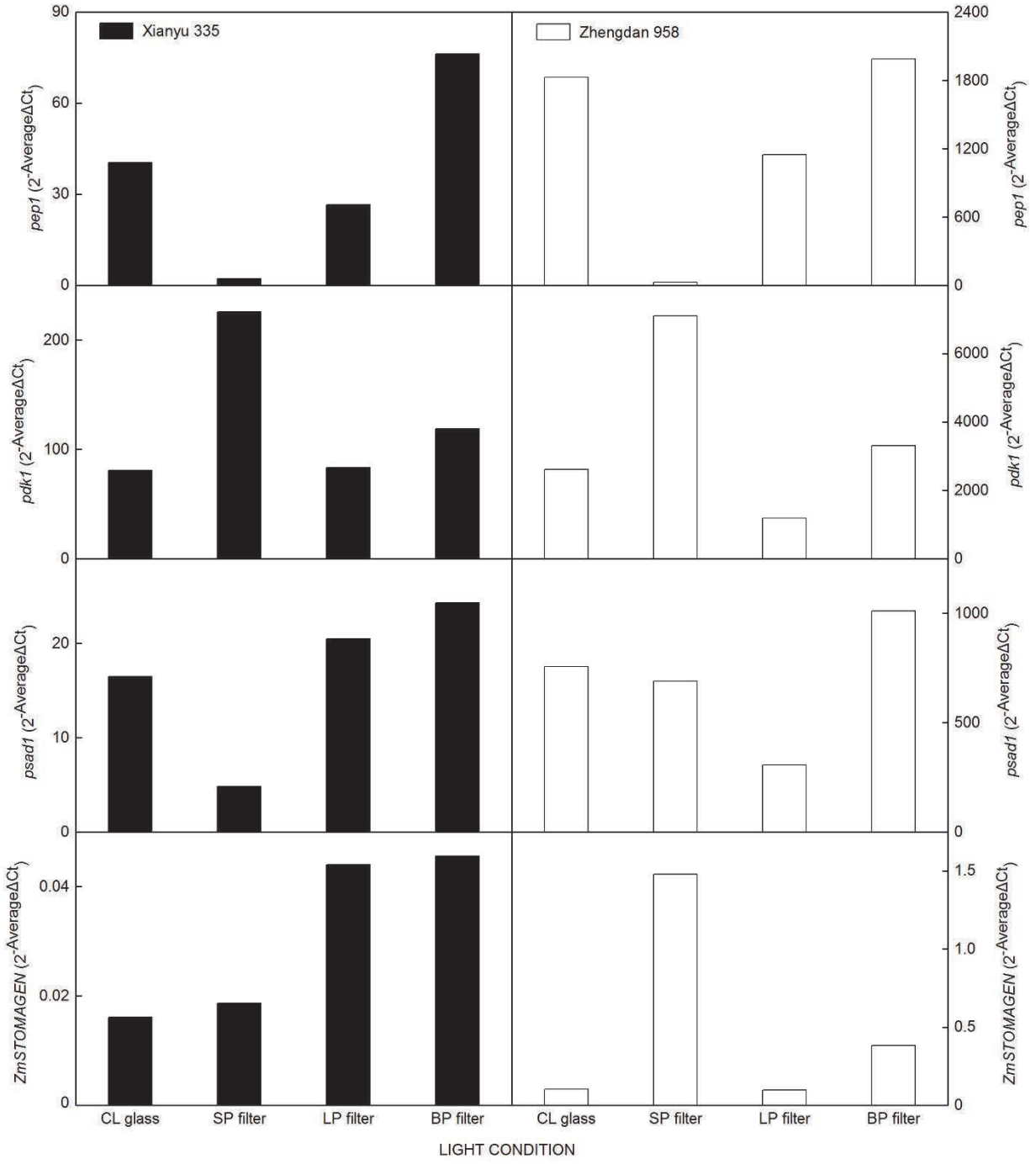

Fig. 1. RT-PCR analysis of *pep1*, *pdk 1*, *psad1*, *ZmSTOMAGEN* expression in response to different light conditions. BP – band pass; CL – transparent glass; LP – long-band pass; SP – short-band pass. Expression results are described as a ratio of the target gene to GAPDH.

filter than that under CL glass. However, the VPD in Zhengdan 958 was significantly higher (20.9%) under the LP filter than under the CL control. Meanwhile, the WUE in Zhengdan 958 was significantly higher by 19.8 and 12.6% under the LP and BP filters, respectively, than those under the CL control. In Xianyu 335, the differences between these parameters followed similar patterns, except that of VPD, which was lower under all three filters compared with CL. However, these differences were generally insignificant.

Maize photosynthesis-related genes were regulated by light conditions: The expression of *pep1*, *pdk1*, *psad1*, and *ZmSTOMAGEN* was studied in response to various

light conditions. Fig. 1 shows that the expression level of *pep1* in Zhengdan 958 and Xianyu 335 were 8.7 and 87.9% greater, respectively, under the BP filter compared with that under CL control. However, the expression level of *pep1* in Zhengdan 958 was 98.3 and 37.1% lower under the SP and the LP filters, respectively, than that under the CL. The *pep1* expression was the lowest under the SP filter. The expression level of *pep1* in Xianyu 335 was 94.2 and 34.4% lower under the SP and the LP filter, respectively, compared with CL. Different expression patterns were observed for *pdk1*. The highest *pdk1* level was observed in Zhengdan 958 under the SP filter, followed by the BP filter. The *pdk1* level was 170.9 and 25.9% higher under the SP and BP filters, respectively, than that under the CL control, but it was 54.5% lower under the LP filter than under the CL. By contrast, the expression levels of *pdk1* in Xianyu 335 were much lower under the different filters; it was 179 and 46.9% higher under SP and LP than that under the CL. The transcript level of *psad1* was 33.9 and 47% greater under the BP compared with CL in Zhengdan 958 and Xianyu 335, respectively; all other treatments showed inconsistent values. The *ZmSTOMAGEN* transcripts were distinctly induced in maize leaves of Zhengdan 958 under the SP filter, whereas the results under the other filters were similar to that under the CL. By contrast, the expression of *ZmSTOMAGEN* was quite low under all light conditions in Xianyu 335 (Fig. 1).

Stomatal size under different light filters: The abaxial surface of our leaf samples were scanned at 400 × magnification. Although individual genotypes were similar in stomatal response to light conditions, close examination revealed significant structural differences between the genotypes (Fig. 2). Both genotypes showed higher stomatal densities under the LP filter, whereas the stomatal density under the SP and BP filters increased by 33.5 and 45%, respectively, relative to that under the CL (Fig. 2). The lengths of stomata (including guard cells) in plants under the BP and SP filters were also altered to a different extent, but remained unchanged under the LP filter. The stomatal width of the both genotypes changed under the SP, LP, and BP filters compared with CL; this parameter decreased in Xianyu 335, but increased in Zhengdan 958 (Fig. 2).

Physiological response: The Chl content was monitored to determine the influence of light conditions as altered by the different filters; the results are shown in Table 2. In Xianyu 335, the Chl *a* and Chl *b* contents were 10.9 and 7.9% higher, respectively, under the BP filter compared with CL. In Zhengdan 958, the content of Chl *a* and Chl *b* contents were 27 and 46.9% higher, respectively, under the BP filter in comparison with CL. The Chl *a* and Chl *b* contents in Xianyu 335 were significantly lower under the SP and LP filters than under CL. However, the Chl *a* and Chl *b* contents in Zhengdan 958 were significantly lower under the SP filter than under CL, but were significantly higher under the LP filter compared with CL. The Chl *a*/*b* ratio was not significantly different under any of the filters compared with CL for both genotypes.

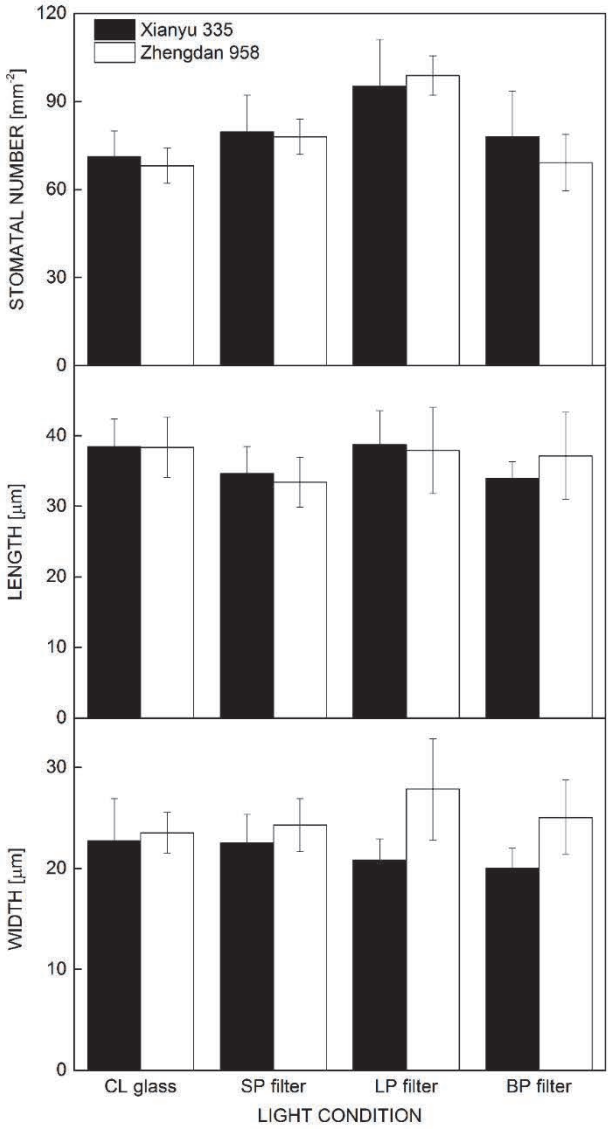

Fig. 2. Stomatal characteristic under different light conditions. BP – band pass; CL – transparent glass; LP – long-band pass; SP – short-band pass.

Table 2. The contents of chlorophyll (Chl) *a*, Chl *b*, and Chl *a*/*b* in leaves under different light conditions. CL – transparent glass; SP – short-band pass; LP – long-band pass; BP – band pass. Data are means ± SD. *Different letters* in rows show significant differences determined by LSD test (p<0.05).

	Xianyu 335				Zhengdan 958			
	SP filter	BP filter	LP filter	CL glass	SP filter	BP filter	LP filter	CL glass
Chl *a* [mg g^{-1}]	8.632 ± 0.197^{b}	9.681 ± 0.170^{a}	7.492 ± 0.137^{c}	8.730 ± 0.006^{b}	5.748 ± 0.179^{d}	8.089 ± 0.173^{a}	7.496 ± 0.134^{b}	6.366 ± 0.167^{c}
Chl *b* [mg g^{-1}]	1.891 ± 0.130^{c}	2.255 ± 0.099^{a}	1.617 ± 0.115^{d}	2.090 ± 0.051^{b}	0.972 ± 0.198^{e}	2.044 ± 0.022^{a}	1.578 ± 0.184^{b}	1.391 ± 0.190^{b}
Chl *a*/*b*	4.594 ± 0.420^{ab}	4.009 ± 0.385^{b}	4.658 ± 0.268^{a}	4.179 ± 0.106^{b}	6.537 ± 1.646^{a}	4.023 ± 0.500^{b}	4.805 ± 0.426^{ab}	4.715 ± 0.801^{ab}

Table 3. The contents of inorganic elements in the leaves under different light conditions. CL – transparent glass; SP – short-band pass; LP – long-band pass; BP – band pass. Data are means ± SD. *Different letters* in volume show significant differences determined by LSD test (p<0.05).

Genotype	Light condition	Mg [mg kg^{-1}]	K [mg kg^{-1}]	Ca [mg kg^{-1}]	N [mg kg^{-1}]	P [mg kg^{-1}]
Xianyu 335	the CL glass	984.95 ± 1.13^{c}	37,844.55 ± 1.97^{d}	2,022.26 ± 2.11^{b}	19,837.76 ± 1.39^{c}	3,798.04 ± 0.11^{d}
	the SP filter	1,690.36 ± 2.18^{a}	45,041.35 ± 3.44^{a}	2,995.98 ± 3.02^{a}	38,002.45 ± 1.88^{a}	7,419.85 ± 0.13^{a}
	the LP filter	998.38 ± 1.07^{b}	44,322.84 ± 5.76^{b}	1,947.13 ± 1.21^{c}	25,028.87 ± 1.05^{b}	5,314.66 ± 0.12^{b}
	the BP filter	897.45 ± 0.84^{d}	39,391.21 ± 5.45^{c}	1,831.08 ± 2.30^{d}	18,232.79 ± 1.62^{d}	5,049.41 ± 0.09^{c}
Zhengdan 958	the CL glass	1,296.22 ± 0.64^{b}	39,394.77 ± 9.45^{d}	2,939.48 ± 1.16^{a}	14,390.06 ± 1.44^{d}	4,418.26 ± 0.09^{d}
	the SP filter	1,468.87 ± 0.97^{a}	44,746.62 ± 5.25^{a}	2,325.09 ± 1.43^{b}	36,091.86 ± 1.74^{a}	6,674.28 ± 0.11^{a}
	the LP filter	1,126.25 ± 0.41^{d}	40,983.48 ± 5.94^{c}	1,958.32 ± 1.92^{d}	20,306.13 ± 1.69^{b}	4,615.63 ± 0.14^{b}
	the BP filter	1,190.44 ± 3.42^{c}	41,402.46 ± 10.43^{b}	2,171.01 ± 1.11^{c}	15,306.07 ± 1.52^{c}	4,484.84 ± 0.11^{c}

Inorganic element contents: In this study, the total concentration of Mg, K, Ca, N, and P in the leaves was determined (Table 3). The Mg, K, N, and P contents were significantly higher by 71.7, 19, 91.5, and 95.3%, respectively, in Xianyu 335 and by 13.2, 13.5, 150.1, and 51%, respectively, in Zhengdan 958 under the SP filter in comparison with CL. Similarly, the K, N, and P contents were significantly higher by 17.1, 26.1, and 39.9%, respectively, in Xianyu 335 and by 4, 41.1, and 4.4%, respectively, in Zhengdan 958 under the LP filter than that under CL. The Mg concentration was the lowest in Xianyu 335 and Zhengdan 958 under the BP filter. The lowest K and P contents in both genotypes were detected under CL. The lowest N concentrations in Xianyu 335 and Zhengdan 958 were observed under the BP and CL filters, respectively.

Discussion

As an initial enzyme of the C_4 photosynthetic pathway, PEPC affects stomatal movement and influences photosynthesis directly or indirectly (Turner and Graniti 1969, Davies 1979, Davies 1980, Schnabl *et al*. 1992). Cousins *et al*. (2007) found that the lack of PEPC decreases stomatal conductance and slows down stomatal opening. In the present study, no relationship was observed between *pep1* and g_s. No correlation was detected between *pep1* expression and photosynthesis in both genotypes. Therefore, we concluded that PEPC was not critical for g_s and photosynthesis under different light conditions (Table 1). The *pep1* overexpression occurred under the LP filter but it might have a restrictive role in photosynthesis. PPDK is the key enzyme in the catalyzing of phospho*enol*pyruvate (Hatch and Slack 1968). This catalytic reaction is mainly regulated by light, which is a limiting factor of the C_4 photosynthetic pathway (Edwards *et al.* 1985). In the leaves of C_4 plants, PPDK is mainly located in the chloroplasts; excessive PPDK expression in *Arabidopsis* could accelerate leaf nitrogen activation in order to accelerate plant growth, increase plant mass and nitrogen content of seeds, and delay senescence (Taylor 2010). PPDK activity is regulated by red and blue part of spectra (Chastain 2011). In this study, *pdk1* was induced by the SP filter. The expression of genes induced under the LP filter was not significant compared with that under CL (Fig. 1). PPDK overexpression obviously accelerated leaf nitrogen (Table 3) under the SP filter. The transcription of PsaD is induced by light (Lotan 1993); PsaD can promote the linear electron transport and polymerization of the PS I reaction center and effectively reinforce the binding of ferredoxin and photosystem. Ca, Fe, and Mg are integral components of ferredoxin; these ions can significantly promote electron transfer in PS (Simon *et al.* 2008). The cryptochrome induced by blue light significantly enhances Mg, K, Ca, and P contents (Yu 2009) (Table 3), but *psad1* did not show increased overexpression under the SP filter compared with CL (Fig. 1). Although the BP extremely promoted the expression of *psad1* as a key gene of PsaD, the content of Mg and Ca ions were quite low. Therefore,

we concluded that light quality rather than inorganic element composition was the decisive factor in PsaD expression. The expression of *ZmSTOMAGEN* was significantly correlated with PPDK (Fig. 1). *ZmSTOMAGEN* was induced under the SP filter; the expression levels of genes induced under the LP filter were not significant relative to those under CL (Fig. 1).

Kim (2004) reported that the stomatal density decreases while the stomatal surface area increases under blue and red light. However, contradictory results were obtained under blue and far-red light. The present results showed that the direct regulation of stomatal development by *ZmSTOMAGEN* was poor under various light conditions (BP, LP, and SP). Although the stomata are only positively regulated during their development, other pathways are still involved in this process. The present results proved that *ZmSTOMAGEN* overexpression in maize under various light conditions cannot significantly promote stomatal development (Fig. 2).

Light quality can modify photosynthesis by adjusting stomata factor, such as stomatal size. In the present study, the size of the stomata under different light conditions was not significantly different between the two maize genotypes. Although the expression levels of PPDK and *ZmSTOMAGEN* were higher under the SP filter, photosynthesis and stomatal development were not significantly increased (except the stomatal density, which was determined early in leaf primodium formation). And the overexpression of maize PEPC and PsaD did not significantly improve photosynthesis as previously reported (Zhang *et al.* 2014). In addition, the stomatal development slightly changed (Fig. 2), and the stomatal conductance shifted, which were contrary to previous reports (Ku *et al.* 1999, Yuan *et al.* 2006). We concluded that the light quality was the most important regulatory factor influencing the expression of photosynthetic genes, however, the overexpression of photosynthetic genes was not the only decisive factors on the photosynthesis. At the same time, although light quality significantly modified the expression of *ZmSTOMAGEN,* which was the only form of positive regulation during the generation of stomata, its overexpression might be strongly offset by other stomatal regulatory pathways.

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