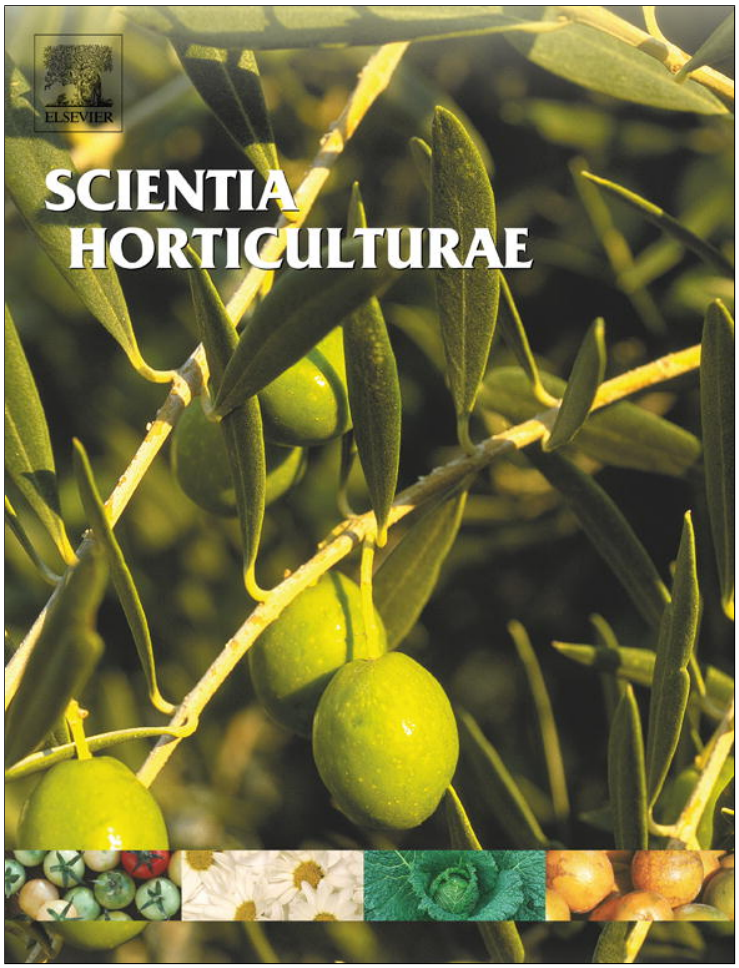

(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Scientia Horticulturae 148 (2012) 190–196

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Applications of xerophytophysiology in plant production—LED blue light as a stimulus improved the tomato crop

Hui-lian Xu[a], Qicong Xu[a], Fenglan Li[b], Yanzhong Feng[c], Feifei Qin[a,d,*], Wei Fang[e]

[a] International Nature Farming Research Center, Hata 5632, Matsumoto-city, Nagano 390-1401, Japan
[b] College of Life Sciences, Heilongjiang Academy of Agricultural Sciences, Harbin, 150086, China
[c] Heilongjiang Academy of Agricultural Sciences, Harbin, 150086, China
[d] Shandong Peanut Research Institute, Shandong Academy of Agricultural Sciences, Qingdao 266100, China
[e] National Taiwan University, Taipei, Taiwan, ROC

ARTICLE INFO

Article history:
Received 24 February 2012
Received in revised form 29 June 2012
Accepted 29 June 2012

Keywords:
Blue light
LED (light-emitting diodes)
Osmotic adjustment
P–V (pressure–volume) curve
Tomato
Plant water relations
Xerophytophysiology

ABSTRACT

Recent research using *Arabidopsis* has shown that blue light stimulates the plant through the receptor protein and induces regulations physiologically and morphologically. It is important to know whether the theory can be used in food crop production. In the present study blue light emitted from light-emitting diodes (LED) was used as a stimulus to tomato crop canopy after sunset for 2 h to induce xerophytophysiological regulations. Blue (450 nm), white and red (660 nm) LED lamps, all with a properties of 0.48 W, 24 V, and 45 μmol m^2 s^{-1} at 10 cm over the lamp, were compared with non-illumination control. A 2 × 4 two factor experiment was conducted in a randomized split block design with the two cultivars as the main block. Results showed that the osmotic potential in the symplasm at full turgor was lower and the leaf turgor potential at full turgor was higher in tomato leaves in blue light treatment. The water fraction in the symplasm in the leaf was larger or the apoplastic water fraction was smaller in leaf of blue light irradiated plants. Both osmotic potential and relative water content at the point of incipient plasmolysis were lower in tomato leaves in blue light treatment. More leaf water was lost by stomatal transpiration and less leaf water was lost by cuticular transpiration in leaves of blue light treated tomato plants. Fruit color was improved and redder in blue light treatment. Fruit yield was increased by all light illumination treatments. The damage of fruit caused by *Helicoverpa armigera* worms was more severely in red light treatment and less severely in blue and white light treatments compared with the control. The leaf blight index was lower in blue and white light illumination treatments than the red light treatment and the control. Both cultivars showed LED illumination responses similar to each other although fruit yield was lower and leaf blight was severer in 'Baiju' than in 'Myoko'. In conclusion, illumination treatment with blue light from LED as a stimulus was effective in fruit yield increase and quality improvement as well as improvement in disease resistance of the tomato crop.

1. Introduction

Plant can perceive its environment changes and transduce the stimulus signal to internal gene systems, where the related genes are activated and transcripted with related enzymes synthesized to catalyze and control biochemical processes for related regulations and adjustments (Mulligan et al., 1997; Yang et al., 1997; Romeis et al., 2000; Smith and Gallon, 2001). Such knowledge has been used to breed plant varieties resistant to adverse environment, such as drought. In research on plant resistance to drought and other adverse environment, scientists usually focus on finding resistant varieties, which are suitable to be grown in adverse environmental conditions. With this knowledge, human has done all this in passive positions to fight against the coming adverse environment. In recent years, scientists have changed their positions to active and tried to induce expected consequences by imposing artificial stimuli to plants. These artificial stimuli include modest drought

Abbreviations: C_{FT}, leaf osmotic solute concentration at fully turgid status; C_S, concentration of solute; CEC, cations exchange capacity; ΔC_{osm}, change in osmotic concentration in symplasm; DI, disease index; LED, light-emitting diodes; *P*, *leaf* turgor potential; P_C, photosynthetic capacity; P_N, net photosynthetic rates; PPF, photosynthetic photon flux; *P–V*, *pressure*–volume; R_D, dark respiration rate; Y_Q, maximum quantum yield; α, constant associated with steep-sloped part of the curve; β, constant associated with the less sloped part of the curve; α', the constant showing the slope of the first steep-sloped phase of the curve; β', the constant related in part with the slope of the second gently-sloped phase of the curve; Ψ, leaf water potential; ζ_{apo}, apoplastic water fraction; ζ_{IP}, relative water content at which leaf turgor reaches zero; ζ_{SC}, relative water content at which stomata are closed; ζ_{sat}, the leaf relative water content at saturation status before drying started; ζ_{sym}, symplastic water fraction; π_{FT}, osmotic potential at a fully-turgid state; π_{IP}, osmotic potential at incipient plasmolysis.

* Corresponding author at: International Nature Farming Research Center, Hata 5632, Matsumoto-city, Nagano, 390-1401 Japan. Tel.: +81 263 926800; fax: +81 263 92 6808.
E-mail addresses: xuhuilian@163.com (H.-l. Xu), jialing_300@163.com (F. Qin).

0304-4238/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scienta.2012.06.044

by restricted irrigation or cultivation practices, partial root drying, hypocotyl exposure, low humidity and rhizosphere salinity. These practices do not necessarily cause real stresses and just induce stimulus signals. In our previous studies, a healthy crop characterized by high leaf turgor potential, high photosynthetic capacity and high seed yield was induced by exposing hypocotyl of peanut (Xu et al., 2009a) or mesocotyl of sorghum (Xu et al., 2009b). Usually, elongation of the hypocotyl of a peanut plant is easily stopped by sun light irradiation through the soil surface crack when the peanut seed is germinating. Consequently the cotyledons remain beneath the soil surface, causing early flowering and early seeds setting. The pods early set on the branches from the cotyledon node compete for carbohydrates with the young plant and the early seeds will rot in soil before harvest. The carbohydrates demand by the early pods would negatively affect the plant growth and, if rotted, contaminate the other pods with aflatoxin (Shen and An, 1988). The problem was solved by inducing peanut hypocotyl elongation by placing more soil over that seed and the elongated hypocotyl was exposed to light by removing the soil over the germinating seed. As early reported and proved by the peanut crop experiment, the hypocotyl is sensitive to light, especially blue light as a component of the strong light. Therefore, it must be interesting if blue light can be a stimulus to induce health consequences of a crop through the so-called blue light response. Plant blue light response was reported as early as 1881 by Darwin after he discovered what is now known as the blue light-induced phototropic response (Darwin, 1881). By the new technology, blue light receptors mediating many photoresponses in plants are found but the details and applications remain elusive until recently. Although detailed signal transduction mechanisms of neither cryptochromes nor phototropins are well understood, significant progress has been made in recent years. However, researches have been done with the model plant of *Arabidopsis* but food plants have not been studied enough without further progress in production technical development. It is well known from the publications, millions of scientists in the world are working on the model plant of Arabidopsis but few work on food plants at the same levels. Therefore, in the present study, blue light was used as an artificial stimulus to irradiate the tomato crop canopy in expectation to induce healthy regulations physiologically and morphologically. LED (light-emitting diodes) was used as the light source. LED lamps were first used in 1960s (Schubert, 2003) in commercial devices as on–off light indicators. Since then, the speed of LED development has steadily increased and LED has become a promising light source used in plant physiology research and thereafter in plant production in enclosed facilities (OIDA, 2002). The combination of red and blue LEDs has proven to be an effective lighting source for several crops (Kim et al., 2005). The addition of 24% green light (500–600 nm) to red and blue LEDs enhanced the growth of lettuce plants compared with plants grown under cool white fluorescent lamps (Kim, 2007). LED is characterized by high potential efficiency in converting electrical power into radiant power, robustness, long life expectancy, small size and directional light emission. The high potential electrical efficiency is an important aspect of LED technology to develop fast, which is expected to contribute to the reduction of global energy consumption and decrease emission of CO_2 (OIDA, 2002). Although the blue light emitted from LED is relatively weak but it represents the component of strong light. The strong light is also one of the drought factors. Therefore, in the present study, as one practice of applications of xerophytophysiology and signal transduction in plant production (Xu, 2007), blue light illumination from LED lamp was used as a stimulus to induce signal transduction rather than light energy supplement. We examined the stimulation-induced osmotic adjustment and the consequent improvements in leaf turgor potential, photosynthetic activities, leaf water retention ability and disease resistance. The final fruit yield increase and fruit quality improvement were discussed in relation with the induced xerophytophysiological regulations.

2. Materials and methods

2.1. Experimental site

Experiment was conducted under rainout shelters in the experimental field at International Nature Farming Research Center in Matsumoto Highland region, Nagano, Japan (E137°52′ N36°12′, 700 m above the sea). The Andosol soil was characterized by a pH of 6, EC of 0.11 mS/cm, total C and total N of 42.0 and 3.2 g kg^{-1}, NH_4—N, NO_3—N, P, K, Ca and Mg of 8.6, 21.6, 88.6, 761, 2561 and 393 mg kg^{-1}, and CEC of 18.8 Meq/100 g. The experimental field has been managed organically without any chemical fertilizer and pesticides for more than ten years.

2.2. Management of the plant materials

Three weeks before tomato seedlings were transplanted, an organic bio-fertilizer (N–P–K = 42–28–18 g kg^{-1}) that was fermented with rice bran, oil mill sludge and fish meal as materials and EM (brand name of a microbial inoculant) as fermentation starter was applied at the rate of 300 g m^{-2}. In the early May, seedlings of two cultivars (Beiju and Myko) of tomato (*Solanum lycopersicum* L.) were transplanted into the rainout shelters, where the site walls of the shelter were rolled up and the environmental conditions were natural without artificial control. The air temperature under the shelter was 5 °C higher and the light was 30% lower than outside. The cultivar Beiju was a large fruit conventional variety and Myoko was a large fruit nature farming variety.

2.3. Experimental design, treatments and statistic analysis

When the fruit setting at the fourth truss was completed and fruit at the first truss start to change color red, LED lamps (Nano Bio Light Technology Co., Ltd., Taibei) of blue, white and red were set under the canopy with two lamps placed above the first truss of fruit on each plant. At sunset, the lamps were turned on and illumination lasted for 2 h. The total illumination period was 3 weeks. Three kinds of LED, blue (450 nm), white and red (660 nm), all with a property of 0.48 W and 24 V, were compared with no-illumination control. The photosynthetic photon flux density was 45 μmol m^2 s^{-1} at 10 cm vertically over the lamp and 15 μmol m^2 s^{-1} at 10 cm horizontally apart from the lamp. A 2×4 two factor experiment was conducted in a randomized split block design with the two cultivars as the main block and four LED irradiations as the sub-block. Each plot included 6 plants and was repeated 10 times. Data were analyzed using the software of DPS Data Processing System (Tang and Feng, 2006). Difference of the means and synergistic interaction between cultivar and illumination were compared according Duncan's new multiple range test.

2.4. Measurement and analysis of photosynthesis

One week after the treatment started, the fully expanded 10th leaf from the uppermost leaf that just emerged was used for measurement of the net photosynthetic rate (P_N) under various photosynthetic photon flux (PPF) from 0 through 250, 450, 600, 800, 1200 and 1600 to 2000 μmol m^{-2} s^{-1}. The temperature and relative air humidity in the leaf chamber were set at 12 °C and 60% respectively with small fluctuations according to the light intensity and transpiration rate. The photosynthetic capacity (P_C), the quantum yield ($Y_Q = KP_C$) and respiration rate (R_D) were analyzed

by model $P_N = P_C\,(1 - e^{-Ki}) - R_D$, where K is a constant and i is the photosynthetic photon flux (Xu et al., 1995).

2.5. Pressure–volume curve analysis

After photosynthesis was measured, the leaf was excised under water, and rehydrated overnight to saturation in a container filled with water in a cool room. The rehydrated excised leaf was used for the pressure–volume (P–V) curve analysis according to Xu et al. (2009a). The P–V curve was modeled as $-\Psi^{-1} = \{\Psi_{FT}^{-1} - \pi_{s+a}^{-1}[\zeta_o - \beta(1-\zeta) - \zeta_{ap}]\}e^{-\alpha(1-\zeta)} + \pi_{s+a}^{-1}[\zeta_o - \beta(1-\zeta) - \zeta_{ap}]$, where Ψ is leaf water potential at a level of relative leaf water content (ζ); Ψ_{FT} is Ψ at full turgor; π_{s+a} is the osmotic potential (π) in the symplastic solution theoretically diluted by apoplastic water; $(1-\zeta)$ is leaf water deficit as the variable in this equation; β is a constant associated with the less sloped part of the curve; α is a constant associated with steeply sloped part of the curve, and ζ_{ap} is apoplastic water fraction and shown as $\zeta_{ap} = \zeta_{FT} - \zeta_{sym} = (1 - \zeta_{sym})$, where, ζ_{sym} is the symplastic water fraction and ζ_{FT} is ζ at full turgor with a value of 1. Since the measurement of P–V curves cost a long time for each sample and the equipment of the pressure chamber was limited, the P–V curve was only measured and analyzed for the cultivars of Myoko, a nature farming variety of tomato.

2.6. Analysis of leaf water retention ability

The leaf water retention curve or so-called transpiration declining curve was analyzed using the fully expanded youngest leaf from the uppermost. The leaf was excised and rehydrated as mentioned above. The rehydrated excised leaf was then placed under light in saturated humidity in a closed apparent acrylic container for 30 min to allow the stomata to open and then the leaves were placed on a net fixed on a light paper box over an electronic balance under 500 μmol m^{-2} s^{-1} of PPF at 60 ± 5% of relative humidity and 22 ± 2 °C of temperature. The leaf sample was recorded at 2–15 min intervals and the water loss on a relative basis was plotted against time. A curve was obtained and modeled by $\zeta = [\zeta_{sat} - \zeta_{SC}(1 - \beta' t)]e^{-\alpha' t} + \zeta_{SC}(1 - \beta' t)$, where, ζ is relative water content of the excised leaf; ζ_{sat} is the leaf relative water content at saturation status before drying started; ζ_{SC} is the leaf relative water content at the time when stomata are closed; t is the time from beginning of the transpiration declining course; β' is the constant related in part with the slope of the second gently-sloped phase of the curve; α' is the constant showing the slope of the first steep-sloped phase of the curve (Xu et al., 2009a). The excised leaves were dehydrated for 9000 s and the leaf relative water content at this time ($\zeta_{9\,ks}$) was also calculated. The transpiration declining curve was only analyzed for the cultivars of Myoko.

2.7. Measurements of leaf color

After photosynthesis was measured, color of the 10th leaf from the uppermost was measured by a portable leaf color meter (SPAD-502, Minolta Co., Ltd., Tokyo, Japan). Color of the relatively lower leaf, the 20th leaf from the uppermost was also measured.

2.8. Measurement of fruit yield

Fruit were harvested from August 10 to October 25. Fruit damaged by *Helicoverpa armigera* (Hübner) and cracking fruit were recorded as mal-fruit.

2.9. Evaluation of disease index

Disease index for leaf blights was estimated as Disease Index (DI) = Σ(number of infected leaves to a certain degree × degree constant)/(total leaf number × highest degree constant). The degree was scored from 0 (no symptom) through 1 (12.5% of the leaf area was infected), 2 (25%), 3 (50%) and 4 (75%) to 5 (completely infected) (Xu et al., 2009c).

2.10. Measurement of fruit color

A master shade guide for red color with five degrees was combined from the MS Word software and printed out on a piece of white paper. The shallowest red was defined quantitatively as 1 and deepest red as 5. The color of fruit was recorded in comparison with the 5° shade guide and the average was calculated.

2.11. Measurement of vitamin C and glucose concentrations

Fruit were sampled and stored in a −85° freezer for measurement uses. Each of the sample fruit was cut into four pieces and one of the upper two pieces was homogenized in distilled water and then the homogenate was infiltrated at 3000 g for 15 min. The supernatant was diluted 400 times for glucose measurement and diluted two times for vitamin C measurement. Both vitamin C and glucose were measured by a reflectometer (RQflex 10, Merck KGaA, Darmstadt, Germany) (http://www.erbsloeh.com/en/datenblatt/WEIN/EasyLab_Manual_englisch.pdf).

3. Results

3.1. Fruit yield and quality

3.1.1. Fruit yield: fruit yield was increased by all light illumination treatments

The fruit yield increase by LED illumination was attributed to increase in fruit size instead the fruit number per plant. There were significant differences ($P \leq 0.01$) in fruit yield and fruit size among LED illumination treatments and the synergistic interaction between cultivar and LED illumination treatments also reached significant level ($P \leq 0.01$). There was no significant difference in fruit number per plant among LED illumination treatments although the difference between cultivars and the synergistic interaction reached significant level of $P \leq 0.01$ (Table 1). Fruit yield in the conventional cultivar (Beiju) was significantly lower than that in the nature farming cultivar (Myoko), which might be due to the severer disease (shown by DI) in Beiju than in Myoko (Table 1).

3.1.2. Fruit color

Fruit color was improved and redder in treatments of blue ($P \leq 0.01$) and white ($P \leq 0.05$) lights but not in the treatment of red light (Table 2). The difference in fruit color reached significant levels ($P \leq 0.05$ or $P \leq 0.01$) among LED illumination treatments and between cultivars at both early and late harvest stages. The synergistic interaction between cultivar and LED reached significant level ($P \leq 0.01$) at the late harvest stage. Fruit color of the cultivar of Beiju was shallower than that of the cultivar of Myoko at the early ($P \leq 0.05$) and late harvest ($P \leq 0.01$) stages (Table 1).

3.1.3. Fruit cracking

There was no significant difference in fruit cracking among LED although the difference reached significant level ($P \leq 0.01$) between the two cultivars and for the synergistic interaction between cultivar and LED treatment (Table 2).

Table 1
Fruit yield and yield components as well as the disease index (DI) in tomato plants treated with different light illuminations.

Cultivar	LED	Yield (g pl^{-1})	Number (pl^{-1})	Size (g)	DI (%)
Myoko	CK	2298^{c}	15.5^{a}	148^{d}	24.9^{c}
	Blue	2599^{a}	15.7^{a}	166^{a}	16.2^{d}
	White	2566^{b}	15.4^{a}	167^{a}	18.3^{d}
	Red	2508^{b}	15.3^{a}	165^{a,b}	23.7^{c}
Beiju	CK	1872^{f}	14.6^{b}	142^{e}	56.8^{a}
	Blue	2075^{d}	14.5^{b}	161^{b}	39.2^{b}
	White	2093^{d}	15.0^{b}	154^{c}	42.4^{b}
	Red	2058^{d}	15.0^{b}	159^{b}	51.6^{a}
Cultivar		**	**	**	**
LED		**	NS	**	**
Cultivar × LED		**	**	NS	**

The data followed by different letters are different from each other at $P \leq 0.05$ and *, ** and NS show the significance of difference or synergistic interaction at $P \leq 0.05$, $P \leq 0.01$ and no existence, respectively, according to Duncan's new multiple range test. The same explanations are used for other tables.

3.1.4. Vitamin C concentration

The concentration of vitamin C in the present study is the concentration of the water soluble L-ascorbic acid. The vitamin C concentration in fruit of tomato plants with blue and white LEDs showed a tendency to be higher than that with the red light of LED. The difference in vitamin C concentration reached significant levels among LED treatments ($P \leq 0.05$) and between cultivars ($P \leq 0.01$). There was no synergistic interaction between cultivar and LED (Table 2).

3.1.5. Glucose concentration

The tomato (*S. lycopersicum*) fruit accumulate the soluble sugars mainly in patterns of glucose and fructose and concentrations of both sugars are proportional to each other in tomato fruit (Yelle et al., 1988). Therefore, the concentration of glucose was used to express the soluble sugar level in the present study. As shown in Table 2, the concentration of glucose was increased by blue and white LED illumination in both cultivars but the red LED showed no this effect. The difference in glucose concentration reached significant levels among LED treatments ($P \leq 0.01$) and between cultivars ($P \leq 0.01$) with a synergistic interaction ($P \leq 0.05$).

3.2. Pest and diseases

3.2.1. Pest insect

Some fruit were damaged by *H. armigera* worms and this damage was more severely in red light treatment and less severely in blue and white light treatments compared with the control (Table 2). The difference in insect-damaged fruit (%) reached significant levels ($P \leq 0.05$ or $P \leq 0.01$) among LED treatments and between cultivars and the synergistic interaction also reached significant level ($P \leq 0.01$).

3.2.2. Leaf blight

Leaf blight was caused by *Alternaria solani* Sorauer. The severity of the leaf blight was shown by the disease index (DI). DI was lower in blue and white light illumination treatments but not lower in red light treatment compared with the control (Table 1). The difference in DI reached significant levels among LED treatments ($P \leq 0.01$) and between cultivars ($P \leq 0.01$) with a significant synergistic interaction between the two factors ($P \leq 0.01$). The reduced DI might be one of the attributes to the improved fruit yield and quality in blue and white LED illumination treatments.

3.3. Photosynthetic activities

3.3.1. Photosynthetic capacity

Photosynthetic capacity (P_C) is the uppermost asymptote in the photosynthesis–light response curve with respiration rate (R_D) added and showed the maximum potential of photosynthesis. P_C was increased by all the three kinds of LED illumination, especially by blue light at later stages. The effect of LED illumination on P_C was equal to the two cultivars without synergistic interaction between cultivar and LED treatment (Table 3). The effect was almost to the same extent from the three LED illumination treatments.

3.3.2. Dark respiration rate

Dark respiration rate (R_D) at both the early and late stages was increased by the illumination from the three kinds of LED with the exception of red LED in the cultivar of Beiju (Table 3).

Table 2
Fruit quality of tomato plants treated with different light illuminations.

Cultivar	LED	Fruit color		Mal-fruit (%)		Vitamin C (mg kg^{-1} FM)	Glucose (g kg^{-1} FM)
		Early	Late	Insect	Crack		
Myoko	CK	4.2^{c}	4.1^{b}	4.6^{d}	1.4^{c}	288.5^{c}	15.2^{c}
	Blue	4.7^{a}	4.4^{a}	2.7^{f}	1.6^{c}	340.2^{a}	16.6^{a}
	White	4.5^{ab}	4.3^{a}	2.2^{g}	1.6^{c}	306.8^{b}	16.2^{a,b}
	Red	4.3^{cb}	4.1^{b}	6.6^{b}	1.5^{c}	280.5^{c}	14.9^{c}
Beiju	CK	4.1^{c}	3.2^{c}	5.3^{c}	2.8^{a}	213.6^{e}	13.8^{c,d}
	Blue	4.7^{a}	3.8^{c}	3.4^{e}	3.2^{a}	242.7^{d}	15.7^{c,b}
	White	4.5^{ab}	3.7^{c}	2.6^{f}	2.7^{a}	250.5^{d}	15.0^{c}
	Red	4.2^{c}	3.3^{d}	7.6^{a}	2.7^{a}	204.6^{e}	13.7^{d}
Cultivar		*	**	**	**	**	**
LED		**	**	*	NS	*	**
Cultivar × LED		NS	**	**	**	NS	*

See Table 1 for the statistic explanations.

Table 3
Parameters from the photosynthetic curve analysis and leaf color of tomato plants treated with different light illuminations.

Cultivar	LED	P_C		R_D		Y_Q		Leaf color	
		(μmol m^{-2} s^{-1})				(10^{-2} mol mol^{-1})		(SPAD)	
		Early	Late	Early	Late	Early	Late	Upper	Lower
Myoko	CK	21.4^{b}	11.2^{d}	2.7^{b}	3.6^{b}	4.49	4.09	54.5^{a}	54.3^{a}
	Blue	24.5^{a}	15.6^{a}	3.1^{a}	4.1^{a}	6.86	6.15	54.2^{a}	53.9^{a,b}
	White	23.6^{a}	12.9^{b}	3.2^{a}	3.9^{a}	6.21	5.61	5.30^{a,b}	53.0^{a,b}
	Red	24.2^{a}	12.8^{b}	3.3^{a}	3.9^{a}	5.73	5.48	53.5^{a,b}	54.2^{a}
Beiju	Control	19.7^{c}	10.1^{e}	2.2^{c}	2.6^{e}	4.78	4.26	52.4^{b}	51.6^{b}
	Blue	21.9^{b}	12.2^{c}	2.7^{b}	3.2^{d}	6.14	5.87	52.7^{a,b}	52.7^{a,b}
	White	21.4^{b}	11.7^{c}	2.6^{b}	2.9^{c}	5.72	5.43	51.0^{b}	52.1^{b}
	Red	21.2^{b}	11.8^{c}	2.3^{c}	2.5^{e}	5.46	4.92	51.8^{b}	52.3^{b}
Cultivar		**	**	**	**	**	**	*	*
Light		**	*	*	*	**	**	NS	NS
Cultivar × LED		NS	**	**	**	**	**	NS	NS

See Table 1 for the statistic explanations.

3.3.3. The maximum quantum yield

The maximum quantum yield (Y_Q) shown by initial slope of the photosynthesis–light response curve and showed the quantum use efficiency, especially important under low densities of photosynthetic photon flux (PPF). Y_Q was increased by illumination from the three kinds of LED, especially by blue light. The difference in Y_Q between cultivars and among LED as well as the synergistic interaction all reached significant levels of $P \leq 0.01$ (Table 3).

3.4. Osmotic adjustment as a xerophytophysiological response

3.4.1. The osmotic potential in the symplasm at full turgor and the difference

Osmotic adjustment is defined as a net increase in solute concentration within a plant cell to maintain a positive turgor pressure by actively accumulating solutes and as a result the osmotic potential drops, promoting the flow of water into the cell. It is not osmotic adjustment if osmotic potential decreases in parallel with the decrease in water content. In the present study osmotic adjustment was analyzed using the pressure–volume curve method. As shown in Table 4, the net increase in solute concentration can be compared between the osmotic potential in the symplasm at full turgor (π_{FT}) and the difference (ΔC_{osm}) can be calculated as the net increase in solute concentration. In the present study, π_{FT} was lower and consequently the leaf turgor potential at full turgor (P_{FT}) was higher in tomato leaves in blue light treatment and then white light treatment than in red light and control treatments. With extents lower than the blue light treated plants, plants treated with illumination of white and red light also showed lower π_{IP} and ζ_{IP} than those in the control plants.

3.4.2. Cell water compartmentation between apoplasm and symplasm

Another consequence of osmotic adjustment is an increase in the water fraction in the symplasm (ζ_{sym}) or a decrease in the apoplastic water fraction (ζ_{ap}) of the cells, where a flow of water from apoplasm into the symplasm was induced by the increases in solute concentration in the symplasm. In the present study, ζ_{sym} was larger or ζ_{ap} was smaller in leaves of blue and white light treated plants (Table 4).

3.4.3. Osmotic potential and relative water content at the point of incipient plasmolysis

Two more parameters analyzed from the *P–V* curve were osmotic potential (π_{IP}) and relative water content (ζ_{IP}) at the point of incipient plasmolysis, when the cell membrane started to separate apart from the cell wall because of water deficit-induced shrink of symplasm. A lower π_{IP} or a lower ζ_{IP} means a high water stress resistance. In the present study, both π_{IP} and ζ_{IP} were lower in tomato leaves in blue light treatment (Table 4). This suggested that plants in blue light treatment plot were more resistant to water stress. Following the blue light treated plants with lower extents, plants treated with illumination of white and red light showed lower π_{IP} and ζ_{IP} than the control plants.

3.4.4. The values of α and β

The constant α showed the slope of the first part in the *P–V* curve. It indicated the easiness of water loss from the cell if the original water amount were the same. The value of α was smaller for the blue light treated tomato plants than for the control plants (Table 4). This meant that water loss from the cell was uneasy in comparison with the control, which might be attributed to the water binding power by the increased solutes in cells. There was no significant difference in α value among blue, white and red light illumination treatments. The value of β in the equation was used to adjust the position up or down and the slope of the second part of the *P–V* curve and it might be associated with the position of the demarcation point between apoplastic and symplastic water fractions. There was also no significant difference in β value among blue, white and red light illumination treatments.

3.4.5. The transpiration declining curve

A group of the excised leaf transpiration declining curves for comparison among different illumination treatments are presented

Table 4
Parameters from the pressure–volume curve analysis for the leaves of tomato plants treated with different light illuminations.

Treatment	Ψ_{FT}	π_{FT}	P_{FT}	π_{s+a}	π_{IP}	ζ_{ap}	ζ_{sym}	α	β	ζ_{IP}	ΔC_{osm} (osmol m^{-3})
	(MPa)										
Control	−0.17^{a}	−0.94^{a}	0.77^{a}	−0.77^{a}	−1.11^{a}	0.18^{a}	0.82^{b}	51.5^{a}	0.99^{a}	0.87^{a}	0.00^{d}
Blue	−0.22^{b}	−1.14^{c}	0.92^{c}	−0.96^{c}	−1.36^{b}	0.15^{b}	0.85^{a}	46.5^{b}	0.98^{a}	0.86^{a}	81.28^{a}
White	−0.22^{b}	−1.11^{c}	0.89^{c}	−0.94^{c}	−1.38^{b}	0.16^{b}	0.84^{a}	44.3^{b}	0.99^{a}	0.84^{a}	71.22^{b}
Red	−0.21^{ab}	−1.05^{b}	0.84^{b}	−0.87^{b}	−1.35^{b}	0.19^{a}	0.81^{b}	43.3^{b}	0.99^{a}	0.85^{a}	43.80^{c}

Improved leaf water retention ability as a xerophytophysiological response.

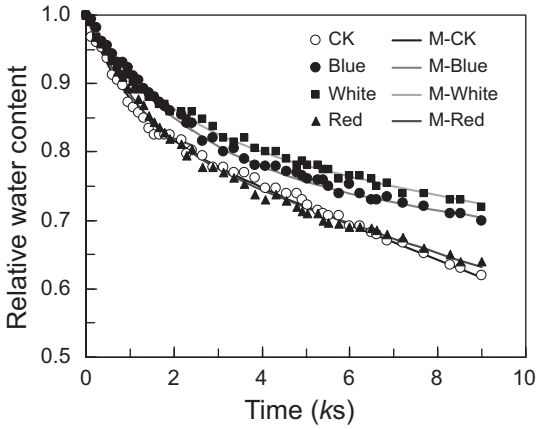

Fig. 1. Transpiration declining curves of the excised tomato leaves treated with different light illuminations.

in Fig. 1. The means of the parameters from the analysis are presented in Table 5. According to the curve shape and the data, the four treatments could be divided into two groups, blue and white as one and red and control as the other. During the whole transpiration or dehydration period, the excised tomato leaves from the treatments of blue and white LED illumination showed higher water retention ability than the red light treatment and the non-illumination control.

3.4.6. Stomatal and cuticular transpiration water loss

The value of the constant α' showed the slope of the first phase in the curve and was associated with the water loss speed by stomatal transpiration, which was lower in treatments of blue and white LED than in red and the control (Table 5). The value of the constant β' showed the slope of the second phase in the curve and was associated with the water loss speed by cuticular transpiration, which was also lower in treatments of blue and white LED than in red and the control (Table 5). From both α' and β', it was suggested that the higher excised leaf water retention ability in blue and white LED treatments was attributed to the lower water loss speed by both stomatal and cuticular transpiration.

3.4.7. The finally retained relative leaf water content after a period of dehydration

The value of $\zeta_{9\,ks}$ showed the integrated leaf water retention ability associated with both stomatal and cuticular transpiration water losses after the excised leaves were dehydrated for 9000 s. As shown in Table 5, $\zeta_{9\,ks}$ was higher in leaves from the treatments of blue and white LED illumination than in red light treatment and the non-illumination control.

3.4.8. Stomatal closure point

ζ_{SC} and t_{SC} showed the leaf relative water content and time needed, respectively, when the stomata in the excised leaves were roughly closed. The lower rate of stomatal transpiration in blue and white LED treated tomato leaves were maintained to lower water content, which was shown by the value of ζ_{SC}, and for longer time, which was shown by the value of t_{SC}. This suggested that the stomatal conductance which was associated with photosynthetic activity in blue and white LED illuminated tomato leaves could be maintained for a longer time and to a severer water deficit in comparison with the non-illumination leaves. The red LED illumination had no this effect.

Table 5
Parameters analyzed from the excised leaf transpiration declining curves.

Plot	ζ_{SC}	t_{SC} (*k*s)	$\zeta_{9\,ks}$	α'	β'
CK	0.837[a]	1.98[c]	0.632[b]	0.998[a]	0.0268[a]
Blue	0.786[b]	3.55[a]	0.697[a]	0.496[d]	0.0122[b]
White	0.818[a,b]	2.88[b]	0.708[a]	0.594[c]	0.0138[b]
Red	0.821[a]	2.16[c]	0.649[b]	0.790[b]	0.0235[a]

ζ_{SC}, relative leaf water content at stomatal closure; $\zeta_{9\,ks}$, relative leaf water content at the time point of 9 ks; α' and β', constants related with stomatal and cuticular transpiration, respectively.

4. Discussion

In our previous studies, artificial stimulation treatments of short-term mild soil water deficits by restricted irrigations to tomato (Xu et al., 2009c), exposition of hypocotyls of peanut or cloves of garlic (Xu et al., 2009a,b) and low air humidity to tomato (Xu et al., 2007) were used to stimulate plants and to induce osmotic adjustment and the consequent improvements in photosynthetic activities, product quality and disease resistance. In the present study, blue light was used as one of the components of strong light, which was in turn as one of the component factors of drought, to induce active osmotic adjustment and the consequent improvement in tomato fruit quality and possibly in fruit yield and pest and disease resistance of tomato crops. As expected, results showed that the solute concentration in the symplasm was increased by blue light stimulation with a lower leaf osmotic potential, the same leaf water potential and a consequent higher leaf turgor potential, which in turn supported a higher leaf photosynthetic activities and the consequent higher fruit yield. The solute concentration in the symplasm was increased and it consequently induced water flow from the apoplasm into the symplasm. This was confirmed by the *P–V* curve analysis, which showed that the water fraction in the symplasm was larger or the apoplastic water fraction was smaller in leaves of blue light treated plants. Water in the symplasm is directly associated with biochemical metabolism and water in the apoplasm (cell walls) is not directly associated with biochemical metabolism (Auge and Stodola, 1990; Patakas and Noitsakis, 1997). As a consequence of xerophytophysiological response, a higher symplastic water fraction is favorable for biochemical metabolism and plant cells with higher symplastic water fraction possess higher physiological activity, especially under adverse conditions such as drought. According to the *P–V* curve analysis, both osmotic potential and relative water content at the point of incipient plasmolysis were lower in tomato leaves in blue light treatment, which suggested that these plants were healthier and more resistant to water stress. The incipient plasmolysis is the extreme crisis of the plant cells under drought or salinity conditions. Osmotic adjustment as abovementioned could allow the plant cells to avoid this crisis up to a severer water stress. It was also an indication of the crop health. Analysis of transpiration declining curve of the excised leaves also confirmed that less water was lost by transpiration in leaves of blue light treated tomato plants, while stomatal conductance was maintained to a severer water stress during leaf dehydration. Following the blue light, white LED light also showed effect of inducing osmotic adjustment and the consequent improvement in plant health and fruit quality to an extent less than or similar to blue light. However, red light did not show the same effect of inducing osmotic adjustment and the consequent improvement in plant health and fruit quality although it also showed the same improvement in fruit yield. Some fruit were damaged by *H. armigera* worms and this damage was more severely in red light treatment, which might be due to the phototaxis of *H. armigera* adults to the red light.

Up to now, there are many research cases done on plant blue light responses. One of the early cellular blue light responses discovered by Spalding and Cosgrove (1992) was the blue light-induced rapid plasma membrane depolarization. This membrane depolarization is caused by the opening of ion channels in response

to blue light stimulation (Cho and Spalding, 1996), where, changes in calcium homeostasis induces the blue light signaling (Christie and Jenkins, 1996; Long and Jenkins, 1998). Blue light may trigger a localized change in the calcium concentration surrounding the nucleus, which in turn affects gene (SUB_1) activity and nucleocytoplasmic trafficking of photoreceptors or photoreceptor signaling molecules (Lin, 2002). Stomatal opening is another movement response mediated by phototropins and the blue light receptor mediating stomatal opening is located in the guard cells (Zeiger and Helper, 1977; Assmann et al., 1985; Zeiger, 2000). It was hypothesized that zeaxanthin might be a candidate chromophore of the photoreceptor (Zeiger, 2000). Guard cells are turgor valves that control the dimensions of the stomatal pore by changes in water content caused by changes in their osmotic potential. In response to light, salt concentration increases in the guard cells, causing an inflow of water, expansion of guard cells, and opening of stomatal aperture. Blue light-specific stomatal opening is mediated by potassium and chloride uptake and malate biosynthesis. Ion uptake is driven by an electrochemical gradient generated by a proton-pumping. Blue light induces phosphorylation of a plasma membrane proton ATPase (Kinoshita and Shimazaki, 1999). The activation of the ATPase is mediated by the phosphorylation of its C terminus by a Ser/Thr kinase, facilitated by the binding of a 14-3-3 protein (Kinoshita and Shimazaki, 1999). The action of H^+-ATPase elevates the inside negative electrical potential gradient across the plasma membrane. This electrical potential gradient drives a voltage-gated K^+ channel, resulting in an accumulation of potassium salt inside guard cells and eventually opening of stomata (Schroeder et al., 2001). In the present study, water flow from apoplasm to symplasm was confirmed by the *P–V* curve analysis but it was not clear whether metabolisms such as abovementioned for the guard cells occurred between the apoplasm and symplasm. The methodology and the related research are needed to further clarify the osmotic adjustment in the cell symplasm.

According to the molecular biological research, cryptochromes are blue light receptors that regulate various photomorphogenic responses in plants, including deetiolation and photoperiodic control of floral initiation (Cashmore, 2003; Lin and Shalitin, 2003). Phytohormones, such as auxin, GA, brassinosteroids, ethylene, and cytokinin, are involved in hypocotyl growth (Vandenbussche et al., 2005; Zhao et al., 2007). Auxin, GA, and ABA change when the etiolated pea seedlings were exposed to light. Many photoreceptor-regulated genes encode enzymes involved in the biosynthesis and catabolism of phytohormones (Zimmermann et al., 2004; Jiao et al., 2005). Although further research is needed to clarify the molecular biological mechanisms for blue light induced osmotic adjustment and the consequent physiological improvements, it is not denying that blue light such as that emitted from LED can be applied in the practical plant production. Especially, the LED costs energy less than the normal lamps. As done in the present experiment, the PPF that the tomato leaves received from the lamp were more or less than 45 $\mu mol\, m^2\, s^{-1}$. The irradiation was just used as a stimulation to induce the expected results and it did not cost much energy, neither the expensive equipment was needed. Moreover, the illumination by the small LED lamps did induce the right expected improvements in plant physiology and the practice was effective in fruit yield increase and quality improvement as well as improvement in disease resistance of the tomato crop. It is concluded that this kind of LED blue illumination is feasible in plant production.

References

Assmann, S.M., Simoncini, L., Schroeder, J.I., 1985. Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba*. Nature 318, 285–287.

Auge, R.M., Stodola, A.J.W., 1990. An apparent increase in symplastic water contributes to greater turgor in mycorrhizal roots of droughted Rosa plants. New Phytol. 115, 285–295.

Cashmore, A.R., 2003. Cryptochromes: enabling plants and animals to determine circadian time. Cell 114, 537–543.

Cho, M.H., Spalding, E.P., 1996. An anion channel on Arabidopsis hypocotyls activated by blue light. PNAS 93, 8134–8138.

Christie, J.M., Jenkins, G.I., 1996. Distinct UV-B and UV-A/blue light signal transduction pathways induce chalcone synthase gene expression in Arabidopsis cells. Plant Cell 8, 1555–1567.

Darwin, C., 1881. The Power of Movement in Plants. D. Appleton and Company, New York.

Jiao, Y., Ma, L., Strickland, E., Deng, X.W., 2005. Conservation and divergence of light-regulated genome expression patterns during seedling development in rice and Arabidopsis. Plant Cell 17, 3239–3256.

Kim, H.H., 2007. Green-light supplementation for enhanced lettuce growth under red- and blue-light-emitting diodes. Hort. Sci. 39, 1617–1622.

Kim, H.H., Wheeler, R.M., Sager, J.C., Yorio, N.C., 2005. Light-emitting diodes as an illumination source for plants: a review of research at Kennedy Space Center. Habitation 10, 71–78.

Kinoshita, T., Shimazaki, K., 1999. Blue light activates the plasma membrane H-ATPase by phosphorylation of the C-terminus in stomatal guard cells. EMBO J. 18, 5548–5558.

Lin, C.T., 2002. Blue light receptors and signal transduction. Plant Cell Suppl., S207–S225.

Lin, C., Shalitin, D., 2003. Cryptochrome structure and signal transduction. Annu. Rev. Plant Biol. 54, 469–496.

Long, J.C., Jenkins, G.I., 1998. Involvement of plasma membrane redox activity and calcium homeostasis in the UV-B and UV-A/blue light induction of gene expression in Arabidopsis. Plant Cell 10, 2077–2086.

Mulligan, R.M., Chory, J., Ecker, J.R., 1997. Signaling in plants. PNAS 94, 2793–2795.

OIDA (Optoelectronics Industry Development Association), 2002. Light emitting diodes (LEDs) for general illumination: an OIDA technology roadmap. In: An OIDA Technology Roadmap Update 2001. OIDA, Washington, DC.

Patakas, A., Noitsakis, B., 1997. Cell wall elasticity as a mechanism to maintain favorable water relations during leaf ontogeny in grapevines. Am. J. Enol. Vitic. 48, 352–356.

Romeis, T., Piedras, P., Jones, J.D.G., 2000. Resistance gene-dependent activation of a calcium-dependent protein kinase in the plant defense response. Plant Cell 12, 803–815.

Schroeder, J.I., Allen, G.J., Hugouvieux, V., Kwak, J.M., Waner, D., 2001. Guard cell signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 627–658.

Schubert, E.F., 2003. Light-Emitting Diodes. Cambridge University Press, Cambridge.

Shen, Y.J., An, K., 1988. Effect of controlling peg growth at the start of groundnut (*Arachis hypogaea* L.) flowering. Oleaginoeux 43, 127–134.

Smith, C.J., Gallon, J.R., 2001. Living in the real world: how plants perceive their environment. New Phytol. 151, 1–6.

Spalding, E.P., Cosgrove, D.J., 1992. Mechanisms of blue light induced plasma membrane depolarization in etiolated cucumber hypocotyls. Planta 188, 199–205.

Tang, Q.Y., Feng, M.G., 2006. DPS Data Processing System – Experimental Design, Statistical Analysis and Data Mining. Science Press, Beijing.

Vandenbussche, F., Verbelen, J.P., Van Der Straeten, D., 2005. Of light and length: regulation of hypocotyl growth in Arabidopsis. BioEssays 27, 275–284.

Xu, H.L., 2007. Xerophytophysiology in crop production. In: Xu, H.L. (Ed.), Dryland Crop Production – Technology Breakthroughs and Study Cases. Research Signpost, Kerala (India), pp. 37–54.

Xu, H.L., Gauthier, L., Gosselin, A., 1995. Effects of fertigation management on growth and photosynthesis of tomato plants grown in peat, rockwool and NFT. Sci. Hort. 63, 11–20.

Xu, H.L., Qin, F.F., Wang, J.S., Xu, R.Y., Wang, M.L., Morita, S., 2009a. Applications of xerophytophysiology in plant production – peanut cultivation with the AnM method. J. Food Agric. Environ. 7 (2), 565–570.

Xu, H.L., Qin, F.F., Xu, R.Y., Wang, F.H., Li FM, 2009b. Applications of xerophytophysiology in plant production – sorghum plants improved by exposing the mesocotyl as stimulus. J. Food Agric. Environ. 7 (2), 603–610.

Xu, H.L., Qin, F.F., Wang, F.H., Xu, Q.C., Wang, R., Shah, S.K., Zhao, A.H., Li, F.M., 2009c. Applications of xerophytophyiology in plant production – partial root drying improves tomato crops. J. Food Agric. Environ. 7 (3&4), 981–988.

Xu, H.L., Iraqi, D., Gosselin, A., 2007. Effect of ambient humidity on physiological activities and fruit yield and quality of greenhouse tomato. Acta Hort. 761, 85–92.

Yang, Y., Shah, J., Klessig, D.F., 1997. Signal perception and transduction in plant defense responses. Genes Dev. 11, 1621–1639.

Yelle, S., Hewitt, J.D., Robinson, N.L., Damon, S., Bennett, A., 1988. Sink metabolism in tomato fruit, III. Analysis of carbohydrate assimilation in a wild species. Plant Physiol. 87, 737–740.

Zeiger, E., 2000. Sensory transduction of blue light in guard cells. Trends Plant Sci. 5, 183–185.

Zeiger, E., Helper, P., 1977. Light and stomatal function: blue light stimulates swelling of guard cell protoplasts. Science 196, 887–889.

Zhao, X.Y., Yu, X.H., Foo, E., Symons, G.M., Lopez, J., Bendehakkalu, K.T., Xiang, J., Weller, J.L., Liu, X.M., Reid, J.B., Lin, C.T., 2007. A study of gibberellin homeostasis and cryptochrome-mediated blue light inhibition of hypocotyl elongation. Plant Physiol. 145, 106–118.

Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L., Gruissem, W., 2004. GENEVESTIGATOR: Arabidopsis microarray database and analysis toolbox. Plant Physiol. 136, 2621–2632.