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Rice yield corresponding to the seedling growth under supplemental green light in mixed light-emitting diodes

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ABSTRACT

The objective of this study was to investigate the effect of different supplemental intensity of green light in mixed light-emitting diodes (LEDs) on rice (*Oryza sativa* L.) seedling growth, and their after-effect on grain yield. The rice seedlings were nursed in greenhouse with 30-days continuous supplemental lighting (6 h/day) from three light sources: 75% red + 25% blue (photon flux density) LED (RB), 62.5% red + 25% blue + 12.5% green LED ($RBG_{12.5}$) and 50% red + 25% blue + 25% green LED (RBG_{25}), and then transplanted into paddy field in two consecutive years (2014 and 2015). The results showed that both shoot and root growth of rice seedlings were enhanced by the addition of green light into red and blue LEDs, but the response of different organ systems depended on the intensity of green light. The low percentage of green light in the light source ($RBG_{12.5}$) could not only promote the stem elongation, the shoot dry weight accumulation and the root respiration activity but also could change the root morphology (indicated by the total surface area and the average diameter of root), while the high percentage of green light (RBG_{25}) only changed the root morphology and increased the root respiration activity. The influence of light on rice during the seedling stage extends to the end of the maturity stage, with the highest rice grain yield, spikelets per panicle and the grain filling percentage in RBG_{25}.

Keywords: green lighting; light intensity; radiation; pigments; productivity

Light, more exactly quality and quantity of radiation, is one of the most essential environmental factors for plant growth and development (Folta and Maruhnich 2007, Jung et al. 2013). In the visible wavelength range (380–780 nm), red light (640–660 nm) and blue light (430–450 nm) were the most absorbed light by chlorophylls and other pigments in the higher plants, while green light (500–600 nm) was the least (Abboud et al. 2013). Therefore, green light was considered as inefficient for photosynthesis in higher plants as compared to red and blue light (Nishio 2000). In contrast, recently increasing researches claimed that green light can lead to adaptive changes in morphology and physiology of plant for optimal growth (Folta and Maruhnich 2007, Terashima et al. 2009, Johkan et al. 2012, Wang et al. 2015). For example, Folta (2004) found that the early stem length of *Arabidopsis* seedlings was increased with green light irradiation. Kim et al. (2004a,b) reported that the stomatal conductance, leaf growth and the dry matter production of lettuce plants were enhanced by the addition of green light into light source. Terashima et al. (2009) indicated that

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green light mixed with strong white light drove photosynthesis more effectively than red light in sunflower leaves. These examples have demonstrated the beneficial effects of green light, but rarely considering the intensity of green light and the response of cereal crops.

Rice, which provides 20% of dietary energy supply in the world, is the dominant staple food in China (Jung et al. 2013). Rice planted in the region of Northeast China is well known for its high eating quality (Zader 2012). Due to the high latitudes in this region, nursery management in greenhouse is needed before rice young seedlings are transplanted into paddy field. The short day lengths and unfavourable conditions of low natural light in greenhouse (Figure 1) are the main limiting factors in nursing vigorous seedlings, which are expected to directly contribute to a plentiful harvest of rice (Pasuquin et al. 2008, Brar et al. 2012). Supplementary illumination was proposed to effectively combat this scourge. However, the information regarding the effects of light on rice seedling growth and its after-effect on rice yield is limited in this region.

Among the various artificial light sources, light-emitting diodes (LEDs) hold the most promise for plant cultivation under controlled-environment conditions because of its small size, long lifetime, low emitting temperature, narrow-bandwidth wavelength emission and wavelength specificity (Massa et al. 2008, Jung et al. 2013). Thus, the objective of this study was to investigate the changes of rice seedling growth under supplemental red and blue LEDs with or without different intensity of green light, and evaluate the effect of light at the seedling stage whether or not it extends to the maturity stage. Hopefully, the findings from this study will be useful to better understand the effects of green light on plant growth, and helpful to design the greenhouse light environment to obtain the vigorous seedlings.

MATERIAL AND METHODS

Site description, plant material and growth conditions. Experiments were conducted in 2014 and 2015 at the Observation Station of Changchun Agroecology (44°00'N, 125°24'E), Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. The mean annual temperature is 4.4°C and the mean annual precipitation is 520 mm. The soil is a typical Black soil (Hapudoll, USDA Soil Taxonomy) with a pH of 7.3, 19.1 g organic C/kg and 1.6 g N/kg.

The rice cultivar planted was Dongdao-4 (*Oryza sativa* L.), which is a Japonica-type elite cultivar. 24-h water-soaked pre-germinated rice seed was manually sown in 72-cell seedling tray (54 cm × 26 cm) at 2016 seeds/m^2 on 5 April 2014 and 8 April 2015. All of the nursery seedling trays were placed on the ground of greenhouse. The air temperature was maintained at 25/15°C day/night cycle, and irrigation at 2–3 days interval until the seedlings were ready for transplanting. When the rice plants fully expanded the first true leaf, 12 sets of randomly selected nursery trays were subjected to supplemental light treatment on 27 April in 2014 and on 25 April in 2015. After 30 days of continuous supplemental lighting, 15 randomly selected seedlings in each treatment were used to determine the morphology of rice. On the same day, all of the seedlings were transplanted to the paddy field at the rate of 3.0 × 10^5 plants/ha^2. Around the plots of light-treated seedlings, metal rods were inserted into the soil and used as the label to differentiate the treatments. At maturity stage (27 September), grain yield and its components were determined. Each year, 83.7 kg N/ha, 55.8 kg P/ha and 105.6 kg K/ha were applied as basal fertilizer. Additional 16.7 and 25.1 kg N/ha were applied as a top dressing at tillering and

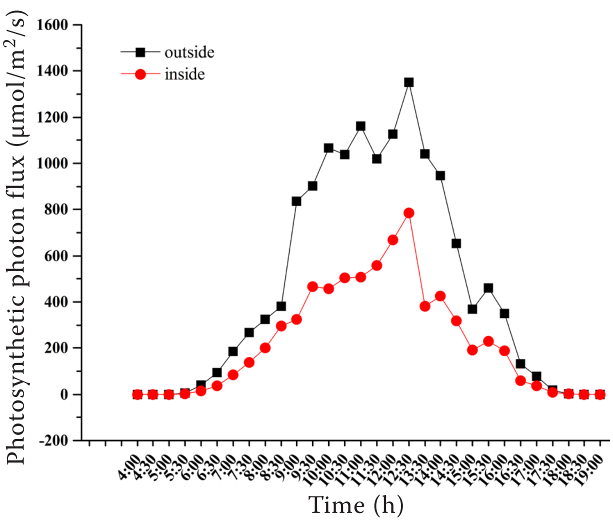

Figure 1. The mean daily value of photosynthetic photon flux on the inside and outside of the greenhouse cover (15 March–15 April, 2014)

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panicle initiation stage. Ponded water depth in the entire field was kept between 1 cm and 3 cm from transplanting to about 7 days before rice harvest.

Supplemental light treatments. Three LED panels (Wetel Lighting Co., Ltd., Shenzhen, China; 55 cm × 28 cm): (1) 75% red + 25% blue (photon flux density) LED (RB, peak wavelength: 662 + 459 nm, bandwidth at half peak height: 19 + 29 nm); (2) 62.5% red + 25% blue + 12.5% green LED ($RBG_{12.5}$, 660 + 452 + 521 nm, 18 + 21 + 38 nm); (3) 50% red + 25% blue + 25% green LED (RBG_{25}, 661 + 450 + 520 nm, 18 + 21 + 36 nm) each with four replicates were used for supplemental light sources and were placed horizontally 30–50 cm above the nursery trays. Means of spectral qualities for each supplemental light treatment are shown in Table 1. Distance between LED panels and plant canopy were adjusted to get the approximately equal photosynthetic photon flux (PPF) at 1000 μmol/m^2/s. Illumination of supplemental light source was for 6 h (from 4:00 to 7:00 am and 4:00 to 7:00 pm) per day in all treatments. The plants without supplemental light sources irradiation were used as the control (CK).

Measurements. The shoot length and stem width were measured with slide callipers. The number of tillers was counted. Tillers with at least one visible leaf were included. The leaf area was determined by using LI-3100 Area Meter (LI-COR, Lincoln, USA). The root morphological parameters were analysed using WinRHIZO software developed by the Regent Instruments Canada Inc. (Ottawa, Canada). For dry weight determination, seedling shoot and root was separately oven-dried at 105°C for 20 min to stop plant respiration, then oven-drying at 80°C till constant dry weight.

Root respiration activity was analysed by the triphenyltetrazolium chloride (TTC) method (Zhang et al. 2013) and expressed as the deoxidization ability (mg/g/h). Briefly, 0.5 g fresh root was immersed in 10 mL of equally mixed solution of 0.4% TTC and phosphate buffer, and kept in the dark at 37°C for 2 h. Subsequently, 2 mL of 1 mol/L H_2SO_4 was added to stop the reaction with the root. The root was dried with filter paper and then extracted with ethyl acetate. The extractant was measured at the absorbance of 485 nm.

Prior to harvest, 20 plants with average panicle number were collected from the centre of each treatment plot to determine grain yield components. Panicles were divided from each sample, hand-threshed, and unfilled spikelets were parted from filled spikelets by submerging them in tap water. Dry weights of filled and unfilled spikelets were determined after oven-dried at 80°C to constant weight. The height of main stems was measured with a meter stick. Grain yield was determined from all remaining plants in each plot excluding those in border rows and converted to kg/ha at 14% moisture content.

Statistical analyses. The ratio of root to shoot was calculated based on the dry mass. To assess the effects of the different supplemental light quality on rice properties, one-way ANOVA test was used, and the least significant difference (*LSD*) was tested. Difference at $P < 0.05$ level was considered to be statistically significant. A linear regression model was calculated to evaluate the relationship between grain yield and the ratio of

Table 1. Summary of the spectral qualities tested for the 75% red + 25% blue light-emitting diodes (LEDs) (RB), the 62.5% red + 25% blue + 12.5% green ($RBG_{12.5}$) and the 50% red + 25% blue + 25% green (RBG_{25})

Parameter	Photon flux (μmol/m^2/s)		
	RB	$RBG_{12.5}$	RBG_{25}
UV-A (350–400 nm)	2.1 ± 0.4	1.6 ± 0.4	1.8 ± 0.5
Blue (400–500 nm)	400.0 ± 25.9 (25.2%)	292.8 ± 13.5 (25.5%)	292.9 ± 21.0 (25.2%)
Green (500–600 nm)	58.8 ± 9.0 (3.7%)	134.1 ± 18.7 (11.7%)	279.1 ± 25.9 (24.0%)
Red (600–700 nm)	1181.0 ± 104.5 (74.1%)	721.2 ± 34.7 (62.3%)	589.5 ± 36.7 (50.8%)
Far-red (700–800 nm)	16.4	11.8 ± 1.4	10.3 ± 1.6
PPF (400–700 nm)	1592.0	1148.0	1161 ± 80.7

The value in parentheses represents the percent over photosynthetic photon flux (PPF). Spectra were recorded 30 cm below the panel of LEDs

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Table 2. Characteristics of rice shoot as affected by providing supplemental lighting from light-emitting diodes (LEDs)

	Treatment	Stem width (cm/plant)	Height (cm/plant)	Number of tillers (per plant)	Leaf area (cm^2/plant)	Shoot dry weight (g/plant)
2014	CK	0.30 ± 0.06^a	17.05 ± 0.98^b	0.31 ± 0.24^a	20.92 ± 1.58^a	0.049 ± 0.014^b
	RB	0.27 ± 0.07^a	17.22 ± 2.44^b	0.24 ± 0.40^a	26.22 ± 6.86^a	0.047 ± 0.012^b
	$RBG_{12.5}$	0.38 ± 0.04^a	21.84 ± 1.81^a	0.49 ± 0.23^a	20.67 ± 4.11^a	0.081 ± 0.021^a
	RBG_{25}	0.30 ± 0.04^a	16.36 ± 1.79^b	0.25 ± 0.18^a	20.67 ± 4.11^a	0.045 ± 0.018^b
2015	CK	0.32 ± 0.03^a	20.23 ± 0.37^c	nd	27.05 ± 1.13^d	0.058 ± 0.009^c
	RB	0.33 ± 0.03^a	23.29 ± 0.27^b	nd	29.01 ± 0.85^c	0.077 ± 0.007^{ab}
	$RBG_{12.5}$	0.34 ± 0.01^a	24.96 ± 0.86^a	nd	33.39 ± 1.28^a	0.084 ± 0.007^a
	RBG_{25}	0.32 ± 0.02^a	22.55 ± 0.29^b	nd	31.35 ± 1.10^b	0.061 ± 0.007^{bc}

CK – without supplemental lighting from LEDs; RB – 75% red + 25% blue LEDs; $RBG_{12.5}$ – 62.5% red + 25% blue + 12.5% green; RBG_{25} – 50% red + 25% blue + 25% green. Values in a column followed by different letters are significantly different at $P < 0.05$; nd – not determined

root to shoot, and the root respiration activity. All statistical analyses were performed by SPSS statistical software (SPSS Inc., Chicago, USA).

RESULTS

Shoot and root characteristics and root respiration activity. The height and the dry weight of shoot were highest in $RBG_{12.5}$ relative to CK and other supplemental light qualities in both years (Table 2, $P < 0.05$). A similar result was also observed for leaf area in 2015 ($P < 0.05$).

Compared with CK, $RBG_{12.5}$ strongly increased root total volume and the number of tips ($P < 0.05$) in 2014 (Table 3, $P < 0.05$). In both years, the higher values of the total surface area and the average diameter were observed in $RBG_{12.5}$ and RBG_{25} than in CK ($P < 0.05$), while the higher total length was only presented in $RBG_{12.5}$ ($P < 0.05$).

Although the dry mass of root was highest in $RBG_{12.5}$ (Table 3), the highest ratio of root to shoot was found in RBG_{25} in both years (Figure 2a,b). Additionally, root respiration activity was higher in $RBG_{12.5}$ and RBG_{25} than that in CK in both years (Figure 2c,d; $P < 0.05$).

Table 3. Root morphology of rice as affected by providing supplemental lighting from light-emitting diodes (LEDs)

	Treatment	Root dry weight (g/plant)	Total length (cm/plant)	Total surface area (cm^2/plant)	Total volume (cm^3/plant)	Average diameter (mm/plant)	Tip number (per plant)
2014	CK	0.013 ± 0.000^b	35.21 ± 2.05	6.38 ± 0.44^b	0.10 ± 0.00^b	0.62 ± 0.01^b	117.46 ± 11.7^b
	RB	0.013 ± 0.004^b	33.09 ± 1.14^b	6.17 ± 0.87^b	0.09 ± 0.03^b	0.59 ± 0.09^b	94.69 ± 8.29^c
	$RBG_{12.5}$	0.024 ± 0.004^a	46.26 ± 2.28^a	10.19 ± 0.96^a	0.19 ± 0.02^a	0.74 ± 0.01^a	139.56 ± 7.10^a
	RBG_{25}	0.013 ± 0.003^b	35.32 ± 0.57^b	7.89 ± 1.20^a	0.15 ± 0.04^a	0.73 ± 0.10^a	91.33 ± 8.78^c
2015	CK	0.023 ± 0.002^b	22.58 ± 2.71^b	4.13 ± 0.53^b	0.24 ± 0.03^a	0.41 ± 0.04^b	67.92 ± 9.3^a
	RB	0.034 ± 0.005^{ab}	25.69 ± 2.56^{ab}	5.13 ± 0.37^{ab}	0.26 ± 0.03^a	0.46 ± 0.08^{ab}	71.33 ± 12.9^a
	$RBG_{12.5}$	0.040 ± 0.008^a	27.19 ± 1.32^a	6.33 ± 0.91^a	0.25 ± 0.05^a	0.50 ± 0.04^a	60.19 ± 17.3^a
	RBG_{25}	0.033 ± 0.003^{ab}	22.92 ± 2.96^{ab}	5.38 ± 0.37^a	0.24 ± 0.02^a	0.50 ± 0.06^a	62.92 ± 3.37^a

CK – without supplemental lighting from LEDs; RB – 75% red + 25% blue LEDs; $RBG_{12.5}$ – 62.5% red + 25% blue + 12.5% green; RBG_{25} – 50% red + 25% blue + 25% green. Values in a column followed by different letters are significantly different at $P < 0.05$

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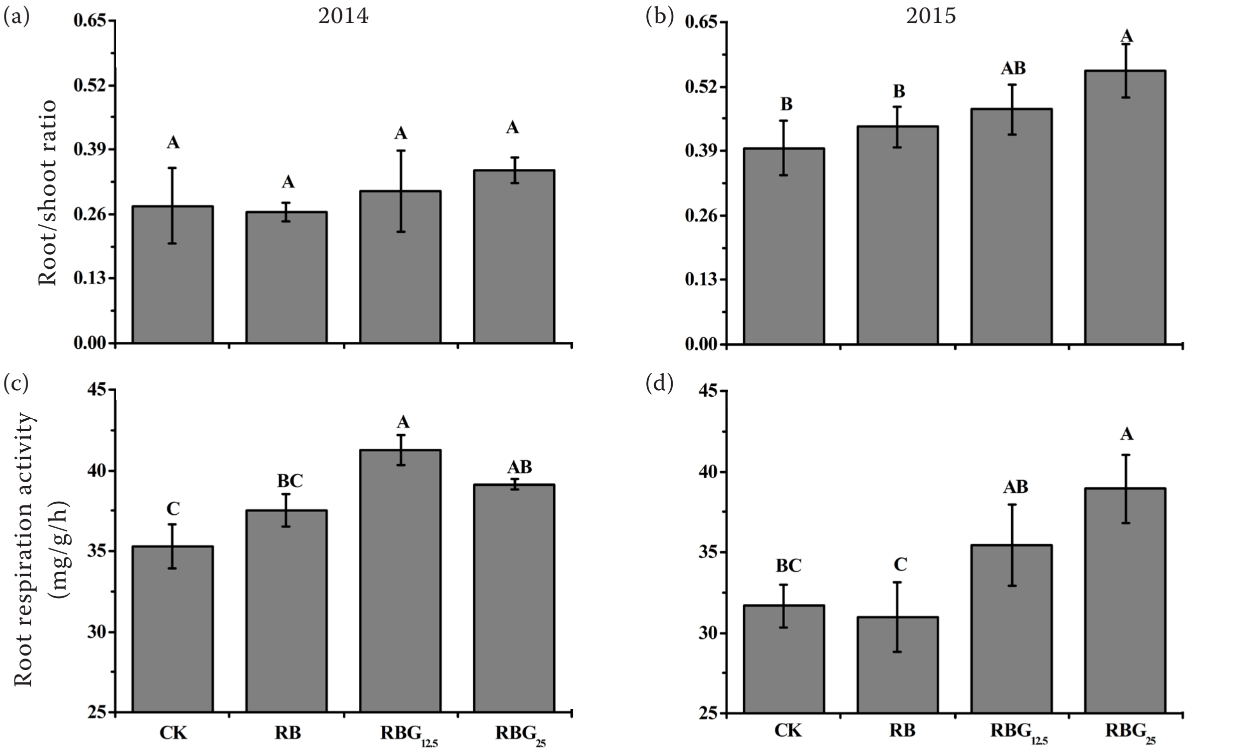

Figure 2. (a, b) The ratio of root to shoot and (c, d) root respiration activity as affected by providing supplemental lighting from light-emitting diodes (LEDs) in 2014 and 2015. Different letters are significantly different at $P < 0.05$

Grain yield and yield components. In both years, the highest value of grain yield was presented in RBG_{25} (Table 4; $P < 0.05$) and followed by $RBG_{12.5}$, RB and CK. A similar trend was also observed for spikelets per panicle and the grain filling percentage in both years. Compared with CK, $RBG_{12.5}$ significantly increased the height of main stems in 2014, and RBG_{25} strongly increased the grain weight in 2015 ($P < 0.05$; Table 4).

Correlations between grain yield and the seedling parameters. Linear regression showed a significant positive correlation between grain yield and the

Table 4. Grain yield and yield components as affected by providing supplemental lighting from light-emitting diodes (LEDs)

	Treatment	Height (cm)	Effective panicles (× 10^4/ha)	Spikelets per panicle (per panicle)	Grain filling percentage (%)	1000-grain weight (g)	Grain yield (kg/ha)
2014	CK	105.5 ± 1.6^a	219.1 ± 23.4^a	114.1 ± 11.3^b	86.6 ± 0.4^b	28.9 ± 1.1^a	8265.6 ± 278.1^b
	RB	104.6 ± 1.2^{ab}	259.1 ± 29.8^a	136.3 ± 10.7^a	89.4 ± 2.6^{ab}	28.7 ± 0.5^a	8971.1 ± 240.8^{ab}
	$RBG_{12.5}$	104.9 ± 1.6^a	248.9 ± 29.3^a	145.8 ± 7.6^a	90.6 ± 0.7^a	27.4 ± 0.2^a	9013.3 ± 796.4^{ab}
	RBG_{25}	102.2 ± 1.1^b	235.4 ± 12.0^a	149.0 ± 9.8^a	91.0 ± 1.1^a	28.8 ± 0.7^a	9182.2 ± 146.9^a
2015	CK	101.7 ± 1.1^a	233.0 ± 17.5^a	88.4 ± 4.7^c	81.6 ± 0.3^c	28.1 ± 0.1^b	7876.1 ± 183.9^b
	RB	100.9 ± 0.9^a	244.5 ± 9.5^a	110.3 ± 1.1^b	83.5 ± 2.0^{bc}	28.9 ± 0.5^{ab}	7779.2 ± 158.8^b
	$RBG_{12.5}$	101.0 ± 0.8^a	255.7 ± 21.0^a	113.5 ± 1.5^{ab}	84.8 ± 1.1^{ab}	29.1 ± 0.7^{ab}	8424.5 ± 450.8^{ab}
	RBG_{25}	100.1 ± 1.6^a	258.4 ± 30.0^a	118.3 ± 5.7^a	86.9 ± 0.2^a	29.4 ± 0.5^a	8930.1 ± 554.8^a

CK – without supplemental lighting from LEDs; RB – 75% red + 25% blue LEDs; $RBG_{12.5}$ – 62.5% red + 25% blue + 12.5% green; RBG_{25} 50% red + 25% blue + 25% green. Values in a column followed by different letters are significantly different at $P < 0.05$

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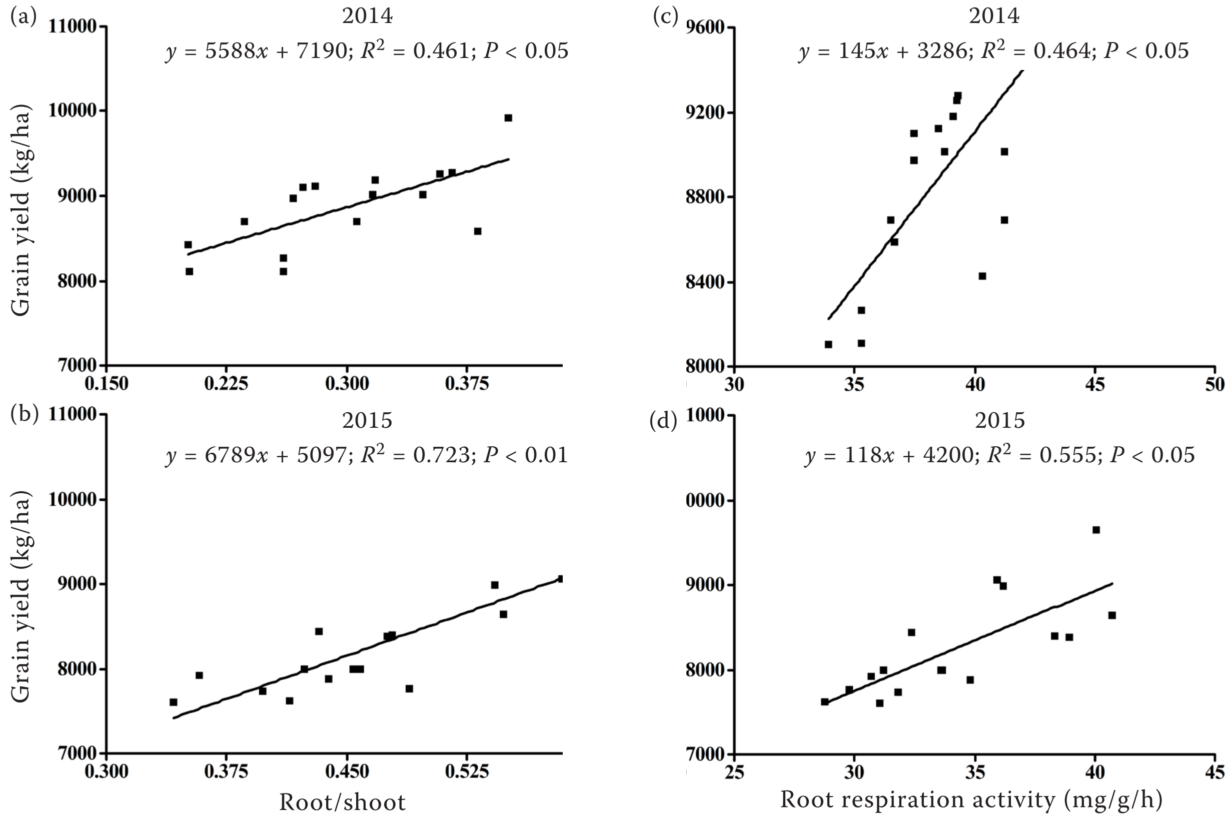

Figure 3. Linear regression between grain yield and (a, b) the ratio of root to shoot, and (c, d) the root respiration activity in 2014 and 2015

ratio of root to shoot (Figure 3a; R^2 = 0.461), the root respiration activity (Figure 3c; R^2 = 0.464) in 2014. Such similar positive correlations (R^2 = 0.723 and 0.555 for root/shoot and root respiration activity, respectively) were also found in 2015 (Figure 3b,d).

DISCUSSION

In this study, as it was expected, the shoot and root growth of rice seedlings were enhanced by the supplemental light; but the performance of young seedlings was different with the different light quality (Tables 2 and 3). A number of studies demonstrated that the combination of red and blue light was an effective light source for plant growth (Lian et al. 2002, Nhut et al. 2003, Matsuda et al. 2004, Jao et al. 2005, Ohashi-Kaneko et al. 2006, Johkan et al. 2010). However, it was found that the highest stem width, shoot and root height, dry weight of shoot and root were all observed in $RBG_{12.5}$ while not in RB. This indicates that the addition of green light to red and blue LEDs is beneficial for stem and root elongation and the dry weight accumulation. Similar result was also reported by Kim et al. (2004a). This might be ascribed to the fact that green light, relative to red and blue light, can better penetrate the plant canopy, which potentially increases plant growth by increasing photosynthesis from the leaves in the lower canopy part (Kim et al. 2004a, Terashima et al. 2009). Alternatively, green light could be reversing the effects of blue wavebands on inhibition of elongation (Bouly et al. 2007, Wang et al. 2015), and therefore the leaves expand more. The effects of green light on enhancing elongation growth were well described in several literatures (Klein et al. 1965, Mandoli and Briggs 1981, Folta 2004, Zhang et al. 2011).

The intensity of light plays a key role in regulating the plant growth (Nhut et al. 2003, Johkan et al. 2012). Kim et al. (2004a) reported that the green photon flux level at 129 μmol/m^2/s strongly decreased the leaf area of lettuce compared with the level at 76 μmol/m^2/s. Similarly, in the present study, neither leaf area nor other measured pa-

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rameters of shoot was found increased in RBG_{25} relative to $RBG_{12.5}$ (Table 2). This suggests that high percentage of green light in the light source may suppress the shoot growth. But, the root growth of rice did not exhibit the same trend as well as shoot. This finding is consistent with what is known about green light effects playing more conspicuous roles in low-light environments (REE) (Zhang et al. 2011, Wang et al. 2015). Although both RBG_{25} and $RBG_{12.5}$ stimulated root respiration activity (Figure 2c,d) and root growth as shown by the increase in total surface area, total volume and average diameter of root (Table 3), the partitioning of biomass between root and shoot (indicated by root to shoot ratio) was highest in RBG_{25} rather than in $RBG_{12.5}$ (Figure 2a,b). This indicates that higher percentage of green light in the light source may allocate a higher proportion of carbohydrates to the root. The results are partially consistent with the reports of Johkan et al. (2012) that the root growth of lettuce was stimulated by the green light with high photosynthetic photon flux (PPF).

Crop yield is always one of the primary objectives in agricultural production. In this study the influence of light on rice during the seedling stage extends to the end of the maturity stage, with the highest rice grain yield, spikelets per panicle and the grain filling percentage in RBG_{25} (Table 4). The observations were in contrast to the findings of Jao et al. (2005) who showed that the difference of *Zantedeschia* plantlets in 28 days of transplant production (*in vitro*) among different LEDs lighting treatments did not last to the end of tuber formation stage grown in greenhouse. By contrast, the observations of this study agreed well with the results reported by Brazaitytė et al. (2009) that the significant effect of different LEDs combination on the tomato seedling remained about one month after moving tomato to greenhouses. These differences may arise from the different light quality, light intensity and plant species. The higher rice grain yield in RBG_{25} relative to CK may be ascribed to the increased root to shoot ratio and the root respiration activity at the seedling stage (Figure 3), which can improve shoot growth by supplying a sufficient amount of nutrients, water and phytohormones to shoots, and subsequently ensure an increase in rice productivity (Ju et al. 2015). Therefore, it is concluded that the effect of lighting treatments on root systems at the seedling stage may affect the later stages of grain development of rice.

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