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BRIEF COMMUNICATION

Responses of tiller growth and related genes expression in rice to red and blue radiation

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Abstract

In the present study, we investigated tiller formation in rice cultivated under red radiation (R), red supplemented with 10 % of blue radiation (RB), and white radiation (W). In addition, expression of three genes related to tiller formation, *OSH1, MOC1*, and *OsTB1*, was analyzed at both vegetative and reproductive stages. RB promoted the outgrowth of tiller buds and increased tiller numbers significantly. Transcription of *MOC1* and *OsTB1* in RB was higher than in R, whereas *OSH1* expression was independent on radiation quality.

Additional key words: laser diode, *Oryza sativa,* real time RT-PCR; spectral composition of radiation.

Recently, it was reported that red radiation (R) supplemented by blue radiation (RB) enhanced light-limited and light-saturated photosynthesis (Matsuda *et al*. 2004), and also that blue radiation (B) significantly increased tillering in wheat plants, which were grown at the same photosynthetic photon flux density (PPFD). As light is a crucial environmental signal controlling various morphogenic and circadian responses in plants (Intrieri *et al*. 2004; Prasad and Zeeshan 2005), we forecast that spectral composition of radiation would have important functions on budding and outgrowth of tillers in rice.

Many important genes related to tillering and flowering had been identified by molecular and genetic studies in rice. *MONOCULM1 (MOC1)* gene was predicted to be a master regulator in controlling of rice tillering, involved in tiller bud initiation and outgrowth (Li *et al*. 2003). *OsTB1* was found to act as a repressor of axillary shoot growth and regulate the sex of the inflorescences terminating the shoot (Takeda *et al*. 2003). Over-expression of *OsTB1* under the control of the actin promoter led to a suppression of tillering in rice (Jackson *et al*. 1994). *OSH1* is considered as a rice counterpart gene of maize *KN1* gene (Sato *et al*. 1996). It has been reported that *OSH1* affects plant hormone metabolism either directly or indirectly and thereby regulates growth in plant development (Kusaba *et al*. 1998).

In this study, we would like to test whether tillering and the related genes are modulated by different spectral composition of radiation. Yamazaki *et al*. (2000) successfully cultivated rice plants by use of laser diodes (LDs) as main radiation sources to realize the rice production in completely closed environment. Based on the achievement, a laser plant factory was built in Hamamatsu Photonics. We cultivated rice plants in the facility under controlled conditions.

Rice (*Oryza sativa* L. cv. Koshihikari) seedlings were grown in soil in a controlled plant factory at Hamamatsu Photonics Central Research Laboratory (air temperature 25 - 30 °C, relative humidity 65 - 70 %, 12-h photoperiod, white radiation 400 μmol m^{-2} s^{-1}) up to three leaves were formed, and then transferred to chambers with different spectral composition of radiation and grown in hydroponics. The plants were grown under red radiation using laser-diodes (LDs) lamps (*L8048*, *Hamamatsu*

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Abbreviations: B - blue radiation; LED - light-emitting diode; LD - laser diode; MHL - metal halide lamp; PPFD - photosynthetic photon flux density; R - red radiation; RB - red supplemented with 10 % of blue radiation; RT-PCR - reverse transcriptase-polymerase chain reaction; SPD - spectral photon-number distribution; W - white radiation.
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Photonics, Hamamatsu, Japan) with the peak emission of 680 nm. Blue radiation was obtained by light-emitting diodes (LEDs) (*NSPB510S*, *Nichia*, Tokushima, Japan) with the peak emission of 475 nm. White radiation was obtained by metal halide lamp (*MT-150-SW, Iwasaki Electric*, Tokyo, Japan). Total PPFD was measured by a quantum sensor (*DataLogger LI-1000*, *Li-COR*, Lincoln, USA).

Samples were collected 2 h before dusk, quickly frozen in liquid N_2 and stored at -80 °C. Total RNA was isolated using RNeasy plant mini kit (*Qiagen*, Hilden, Germany), and treated with DNase to remove contaminating genomic DNA using RNase-free DNase set kit (*Qiagen*) according to the manufacturer's protocols. Tissue was ground to a fine powder, then 50 mg sample was used for RNA extraction and about 20 µg of RNA were extracted. Total RNA concentration was measured at wave length 260 - 280 nm using a spectrophometer (*UV-140-02*, *Shimadzu*, Tokyo, Japan) and examined by 1 % agarose gel electrophoresis. RNA was reverse transcribed to first-stand cDNA using Taqman® Gold RT Kit (*Applied Biosystems*, Foster City, USA) using the following program: 10 min at 25 °C, 30 min at 50 °C and 5 min at 95 °C. The transcripts was confirmed and quantified by PCR (*7500 Real-Time PCR System*) using *Sybr*®Green PCR master mix kit according to the manufacturer's instructions (*Applied Biosystems*). Primers used were:
5'-CTCAAGGTAAACAACAAGGCACA-3' and
5'-GCGCAACGCAAAAGTTAGGC-3' for *OSH1*,
5'-ACTGGCCTCGAGTTTCACCC-3' and
5'-CATGGCCTTCACCCACTTCA-3' for *MOC1*,
5'-GCCGGATGCAAGAAATC-3' and
5'-TCAGCAGTAGTGCCGCGAA-3' for *OsTB1*,
5'-AACCAGCTGAGGCCCAAG-3' and
5'-ACGATTGATTTAACCAGTCCATGA-3' for *ubq*.

The predicted size of the amplified fragment was 123 bp for *OSH1*, 114 bp for *MOC1*, 169 bp for *OsTB1* and 77 bp for *ubq*. 1 mm^3 of the resultant cDNA sample was used as a template for the Q-PCR reaction, with 10 min at 95 °C, 40 cycles of 15 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, then 1 cycle of 10 min at 72 °C.

We compared tillering among those plants cultivated from the initiation of the third leaf opening under white radiation (W), red supplemented with 10 % of blue radiation (RB), and only red radiation (R). Tiller buds were all normally formed at the axils of the four-leaf stage under all radiation condition. However, the buds grew out to tillers under RB and W, while those formed under R remained dormant. This result, therefore, suggests that blue radiation in RB might promote outgrowth of tillers. We then measured the tiller numbers from four-leaf to eight-leaf stage (Table 1). Rice under R had only one tiller when the eighth leaf opened, whereas rice under RB had more than three tillers. In addition, plant under R was more slender and thin compared with plant under RB and W. The results suggest that B might promote tiller growth throughout the vegetative stage. In the reproductive stage, rice plants under R and W still formed fewer tillers than those under RB (Table 1). The final number was fourteen and thirteen under R and W respectively, but almost twenty under RB.

To test the genetic mechanisms involved in the radiation regulated tiller formation and outgrowth, we measured transcription of *OSH1, MOC1* and *OsTB1* in axillary buds at four-leaf and eight-leaf stages (Table 2). The amount of transcripts was quantified by real-time RT-PCR using the *ubiquitin* gene (*ubq*) as reference. Transcription of *OSH1* gene in axillary buds did not differ very much among R, RB and W, which indicated that spectral composition of radiation did not affect *OSH1* and

Table 1. Effect of spectral composition of radiation on tiller numbers at vegetative and reproductive stages. The number of tillers was determined on the indicated leaf on the main culm. Means ± SE, n = 20.

	Vegetative				Reproductive				
	5	6	7	8	8	9	10	11	12
RB	0.15 ± 0.35	1.65 ± 0.47	2.19 ± 0.50	3.10 ± 0.53	4.00 ± 0.55	6.20 ± 0.60	10.80 ± 0.87	18.00 ± 1.78	19.20 ± 2.06
R	0	0.45 ± 0.48	0.75 ± 0.43	1.00 ± 0.54	4.00 ± 0.71	6.60 ± 0.66	9.20 ± 1.02	12.50 ± 1.16	14.20 ± 1.36
W	0	1.08 ± 0.43	1.70 ± 0.55	2.50 ± 0.59	4.00 ± 0.45	6.50 ± 0.50	9.00 ± 0.63	12.10 ± 0.83	13.50 ± 0.92

Table 2. Relative quantitation transcription rate of genes *OSH1, MOC1, OsTB1* in plants grown under W, RB and R. Axillary buds were harvested 2 h before dusk, after complete opening of the indicated leaf. 10 plants of each stage were subjected to real-time RT-PCR analysis.

	4^{th} leaf			8^{th} leaf			12^{th} leaf		
	W	RB	R	W	RB	R	W	RB	R
OSH1	1.0 ± 0.20	1.35 ± 0.10	1.30 ± 0.16	2.0 ± 0.30	1.81 ± 0.26	1.45 ± 0.35	1.4 ± 0.13	1.87 ± 0.27	1.67 ± 0.21
MOC1	1.0 ± 0.17	2.40 ± 0.10	0.76 ± 0.18	0.4 ± 0.17	2.64 ± 0.21	2.75 ± 0.179	1.2 ± 0.26	2.10 ± 0.35	0.70 ± 0.18
OsTB1	1.0 ± 0.13	1.20 ± 0.15	0.45 ± 0.12	1.3 ± 0.17	0.26 ± 0.15	0.56 ± 0.13	4.3 ± 0.12	5.70 ± 0.18	1.30 ± 0.24

the cellular differentiation to form axillary meristems. The transcription of *MOC1* was reduced under W and R at four-leaf stage compared to eight-leaf stage, but kept almost the same under RB. The transcription of *OsTB1* at eight-leaf stage was nearly three times higher than at four-leaf stage under RB, however almost the same as at four-leaf stage under W and R. These results indicated that B promoted the expression of *OsTB1*. We also measured transcription of *OSH1, MOC1* and *OsTB1* in axillary buds in twelve-leaf stage (Table 2). *OSH1* were slightly increased depending on the progress of the leaf stage, but the spectral composition did not affect *OSH1* transcription also in reproductive stage. *MOC1* under RB were three-fold higher than under R and W at the twelve-leaf stage. *OsTB1* expression under RB was almost four-fold higher than under R at this stage. These results indicate that B also promoted transcription of *MOC1* and *OsTB1* in the reproductive stage. Even after flowering and spike production, rice grown under RB still produced many small tillers, which indicated that the plants under RB retained the capacity of tiller bud formation and outgrowth even after transition to the reproductive stage.

From our study, it was shown that spectral composition of radiation affected tiller bud outgrowth at the first node on the main culm. It is obvious that B stimulated the growth of tiller buds, and tillers under RB grew more quickly than those under W. Our result shows that expression of *OSH1* was not affected, whereas both genes of *MOC1* and *OsTB1* were affected by spectral composition of radiation and had higher expression under RB. We suggested that both genes *MOC1* and *OsTB1* were affected by B directly or indirectly, though the signal cascade is still unknown in detail. At the end of the vegetative stage, rice plants undergo transition to the reproductive stage. It seems that rice at the transition stage should produce fewer tillers and concentrate resources for flowering and grain production. Rice under RB produced significantly more tillers from the eight-leaf to the twelve-leaf stages than that under W. We speculate that 5 % of far-red (700 - 750 nm) included in W would be the cause.

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