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Light Quality during Seed Imbibition Affects Germination and Sprout Growth of Soybean

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ABSTRACT : Soybean seeds were treated with blue, red and far-red lights for 0, 6, 12, and 24 hours during 24-hour imbibition before culture for 6 days. The soybean sprouts raised were classified by their hypocotyl lengths; normal (>4 cm), abnormal (<4 cm) and non-germination, and their lateral roots, hypocotyl diameters and component dry weights were measured. Red light treatment and dark imbibition reduced the abnormal soybean sprouts more than far-red and blue light treatments, meaning that the former treatments produced more commercial sprouts. The lateral roots were more formed in blue light and dark imbibition than the other light treatments, but were completely blocked by any light treatment lasted during the whole imbibition. Although any light quality treatment did not influence their primary root lengths, blue light one lengthened the hypocotyl more than the others treated during the imbibition, and far-red light enlarged its diameter. Despite this morphological change, component, total or economic yield was not significantly different among the light quality treatments during the imbibition.

Keywords : Soybean (*Glycine max* L.), Sprout, Light quality, Germination, Growth, Morphology.

Light is the ultimate source of energy for all organisms on the earth. For plants, light supplies the energy for photosynthesis and signals for photomorphogenesis, the ability of light to regulate their growth and development including their seed germination. The former is a reaction to convert it to chemical energy like photosynthates but the latter is one to induce the responses including germination and many morphological characters and to partition the energy to their organs such as stem, leaves, roots, etc. Therefore, nowadays many scientists are considering the latter reaction, photomorphogenesis, as important as the former, photosynthesis (Cosgrove, 1994; von Arnim *et al.*, 1996).

The photomorphogenesis is controlled by a plant pigment, phytochrome consisting of two forms of phytochrome red (Pr) and phytochrome far-red (Pfr). The two phytochromes are interconverted by treatment of red or far-red light so that their ratio can be controlled. The phytochrome mediates the responses such as germination, hook formation and elongation of hypocotyl and internode, initiation and growth of roots and leaves, etc (Cosgrove, 1994; Wareing *et al.*, 1981). Because the above responses, however, are amplified by blue light when Pfr is relatively higher than Pr (Bewley *et al.*, 1982; Kim *et al.*, 1982; Mohr, 1994), artificial red or far-red light treatment to adjust their ratio and blue light treatment to amplify the phytochrome-mediated reponses are necessary for production of good quality soybean sprouts (Kigel *et al.*, 1990; Kim *et al.*, 1982; von Arnim *et al.*, 1996).

Soybean sprouts are one of the most favorite foods for Oriental including Korean. For the reason, a great deal of sprouts is being raised in Korea. At least two problems must be encountered in their culture; decay of non-germinated seeds or weak sprouts and generation of lateral roots (Kang *et al.*, 1989). Their producers, therefore, reduce their decay as spraying fungicides harmful to human being or enhancing the germination rate, and treat the growth regulators to block the appearance of lateral roots. Because using the fungicide is now prohibited, elevating the germination rate is only a method to prevent decaying due to lower germination. Their lateral roots usually appearing after 5 to 6 days can be blocked as soaking the seeds into 4 ppm of benzylaminopurine (BA) solution during 24-hour imbibition (Kang *et al.*, 1996). However, BA is so expensive that their production cost increases. An alternative method to solve the problems must be established in near future.

Light-mediated phytochrome as mentioned above controls the germination as well as the initiation or formation of lateral roots. It is, however, known that the ratio of Pfr to Pr can be much affected by light color and duration, and the phytochrome action can be interacted with growth regulators like BA (Barro *et al.*, 1989). How artificial light treatment and its duration under modified imbibition of BA solution influence seed germination, sprout growth and morphology must be checked for production of good quality soybean sprouts. This study was, therefore, done to examine the effect of artificial light quality treated during the 24-hour imbibition on seed germination, sprout growth and morphology of soybean.

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MATERIALS AND METHODS

Test seed preparation

Test seeds of soybean (*Glycine max* L.) were obtained from Kyungnam Agricultural Research and Extension Service. After debris mixed with them were removed, small and large seeds were eliminated by a sieve shaker. The sorted seeds then were placed into plastic bottles held at 3°C refrigerator until initiating the experiments.

Culture

Plastic containers used for the sprout culture were opaque and rectangular with 9.5 cm in length, 8.5 cm in width and 13 cm in depth. Many holes on their bottoms were made for water drainage. The containers were placed in the dark growth chamber set to 25°C constant temperature after 150 seeds treated were placed into each container. The sprouts were cultured for 6 days. During that time, about 100 ml distilled water adjusted to 25°C was supplied to each container every 6 hours and black fabrics were covered with a sheet of black plastic film to minimize the effect of light given inevitably during water supply.

Treatments

Two cultivars, Eunhakong and Hannamkong, were used as test varieties. To prevent imbibition injury to water, the seeds were soaked for 5.5 hours every 6 hours. This procedure was repeated 4 times. During the final imbibition, however, they were soaked into 4.0 ppm BA solution instead of distilled water to block the formation of lateral roots. Treatments were separated by light quality and its duration. Light quality treatments were forced with blue, red and far-red light using glass filters or darkness. The glass filters produced by Melles Griot Co., USA were inserted in commercial halogen lamps of 5 cm in diameter. Their spectra and intensity were shown in Fig. 1. The quality treatments were done for 24, 12, 6 hours before culture or non. The two treatment factors having 4 levels individually were completely combined to draw their interaction effect.

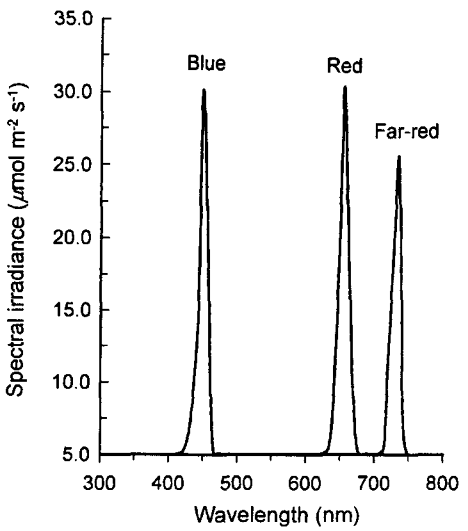

Fig. 1. Light spectrum used for light quality treatment. Measurement was done by the LI-1800 spectroradiometer (LI-COR Co., USA).

Measurement

Number of germination and non-germination seeds, length of hypocotyl and primary root, diameter of hypocotyl hook and its middle part, number of lateral root, and dry weight of cotyledon, hypocotyl and roots were examined or measured after the sprouts cultured for 6 days were divided into 4 groups on the basis of hypocotyl length and germination; longer than 7 cm (>7 cm) and 4 to 7 cm (4~7 cm) as normals, shorter than 4 cm (<4 cm) as abnormals and non-germinated seeds. Non-germinated seeds and growing sprouts were counted for total seeds but the other characters were measured with 20 sprouts selected from above each category. After the selected sprouts were separated into cotyledons, hypocotyl and roots, they were desiccated for 2 days at 70°C to measure their dry weights.

Statistical analysis

Among the characters of soybean sprouts, lateral roots emerged from the conjunction part of their hypocotyl and primary root were important as well as the germination rate. Therefore, the germination rate was first analyzed on the basis of hypocotyl length, the treatments inducing lateral roots were eliminated from the data analysis because any sprout already forming them did not have the commercial value.

RESULTS AND DISCUSSION

In the germination and hypocotyl growth measured on the 6th day after the treatments of light quality for different durations during 24-hour imbibition, the two cultivars, cv. Eunhakong and Hannamkong, showed the significant difference only in the ratios of hypocotyl of A class (>7 cm), or B class (4~7 cm). Summation of A and B in each cultivar, however, was same because cv. Hannamkong had the higher ratio in A class but vice versa in B class. Red and dark treatments showing nearly the same ratios in all the categories resulted in

Table 1. Effect of light quality and treatment duration during 24-hour imbibition on germination and growth of soybean sprout.†

Parameters	Normal		Abnormal	Non-germ	A+B	C+D
	>7 cm (A)‡	4~7 cm (B)	<4 cm (C)	0 cm (D)		
	%					
Cultivar						
Eunhakong	53.7	27.8	11.1	7.4	81.5	18.5
Hannamkong	58.7	22.3	11.8	7.3	81.0	19.0
$LSD_{.05}$	2.3	2.1	ns	ns	ns	ns
Light quality during imbibition						
Red	57.9	25.0	10.0	7.2	82.8	17.2
Far-red	53.1	26.6	13.0	7.2	79.8	20.3
Blue	56.8	22.8	12.0	8.3	79.7	20.3
Dark	56.8	25.8	10.8	6.5	82.7	17.3
$LSD_{.05}$	3.2	3.0	2.0	ns	2.5	2.5
Duration of light treatment (hrs)						
0	56.8	25.8	10.8	6.5	82.7	17.3
6	56.1	23.5	12.1	8.3	79.6	20.4
12	56.0	26.1	10.6	7.3	82.6	17.9
24	55.7	24.8	12.3	7.1	80.6	19.4
$LSD_{.05}$	ns	ns	ns	ns	2.8	2.7

†After three time of drying for 0.5 hour at 25°C following 5.5 hour water imbibition, seeds were soaked in 4.0 ppm BA solution.
‡Hypocotyl length of soybean sprouts cultivated for 6 days after the above imbibition.

greater A plus B ratio than far-red and blue but reverse in C plus D. This result was due to that far-red or blue light treatment decreased the ratio of A or B classes, respectively, compared to red and dark. In duration effect of light treatment during 24-hour imbibition, darkness and 12-hour light treatment at the end of the imbibition had the highest A plus B ratio although there was no significant difference among the duration treatments in each class (Table 1).

The lateral roots important in the commercial cultivation of soybean sprouts were more formed in Eunhakong than in Hannamkong. Blue light and dark treatments in Eunhakong but only dark treatment in Hananmkong stimulated the formation of lateral roots. 24-hour illumination in Eunhakong but all the light treatments in Hannamkong regardless of the duration completely blocked the formation of lateral roots. In two cultivars, therefore, lateral roots were not formed only in full illumination during 24-hour imbibition (Fig. 2), implying that the light treatment during the imbibition could somewhat alleviate their formation.

Only 24-hour light treatment not forming the lateral roots was analyzed to compare the hypocotyl length and diameter. Eunhakong had shorter hypocotyl and primary root length but thicker hypocotyl diameter than Hannamkong in general. Light quality treatment highly influenced only the growth of hypocotyl. Compared to red and far-red lights, blue light lengthened the mean hypocotyl length and aftermath the mean total length adding the primary root length to it. Far-red light, however, induced thicker diameter in all hooks and hypocotyls (Table 2). The light quality treated during 24-hour imbibition did not changed the growth of soybean sprouts but modified their morphological characters such as

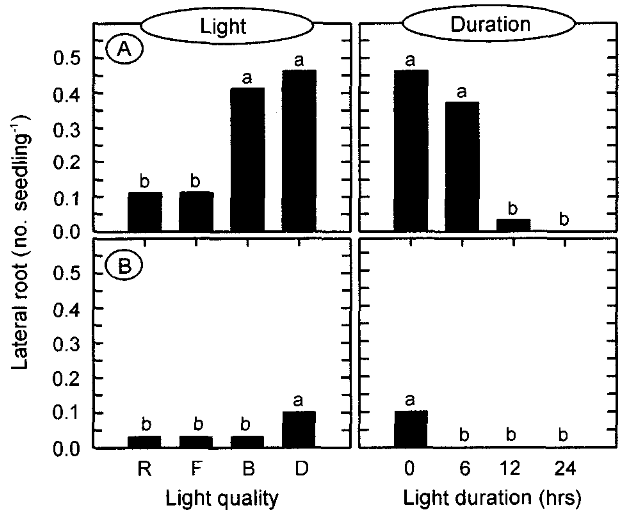

Fig. 2. Effect of light quality (left-sided) and light treatment duration (right-sided) on number of lateral roots of soybean sprout. A on the tops and B on the bottoms indicate cv. Eunhakong and Hannamkong, respectively. Letters on the left-side of X axis indicate R, red; FR, far-red; B, blue light and D, dark. Bars having same letter within the 4-paired treatments were not significantly different from $LSD_{.05}$.

Table 2. Effect of light treatment lasted during 24-hour imbibition on length of hypocotyl and root and hypocotyl diameters of soybean sprout.†

Parameters	Length									Diameter					
	Hypocotyl			Root			Total			Hypocotyl			Hook		
	>7‡	4~7	Mean	> 7	4~7	Mean	> 7	4~7	Mean	> 7	4~7	Mean	> 7	4~7	Mean
	cm sprout^{-1}									mm sprout^{-1}					
Cultivar (C)															
Eunhakong	11.3	6.0	9.6	1.9	1.3	1.7	13.1	7.3	11.3	2.0	2.3	2.1	2.0	1.9	2.0
Hannamkong	11.5	5.8	10.4	3.0	1.6	2.7	14.4	7.4	13.2	1.8	1.8	1.8	2.0	1.9	1.9
$LSD_{.05}$	ns	0.1	0.3	0.2	0.1	0.2	0.4	ns	0.4	0.1	0.1	0.1	ns	ns	0.05
Light quality during 24-hour imbibition (L)															
Red	11.0	5.7	9.6	2.3	1.5	2.1	13.3	7.2	11.7	1.9	2.0	1.9	1.9	1.8	1.9
Fared	10.4	6.2	9.3	2.5	1.3	2.2	12.9	7.5	11.4	2.0	2.1	2.0	2.0	2.1	2.0
Blue	12.3	6.0	11.0	2.5	1.5	2.3	14.9	7.5	13.3	1.9	2.0	1.9	1.9	1.7	1.9
$LSD_{.05}$	0.4	0.2	0.3	ns	ns	ns	0.6	ns	0.5	0.09	ns	0.09	0.1	0.1	0.07
C × L	**	**	**	ns	**	ns	**	**	**	**	*	**	ns	ns	*

†After three time of drying for 0.5 hour at 25°C following 5.5 hour water imbibition, seeds were soaked in 4.0 ppm BA solution.
‡Hypocotyl length (cm) of soybean sprouts cultivated for 6 days after the above imbibition.

Table 3. Effect of light quality lasted during 24-hour imbibition on component and total dry weights of soybean sprout.†

Parameters	Cotyledon			Hypocotyl			Root			Total			Economic yield		
	>7‡	4~7	Mean	> 7	4~7	Mean	> 7	4~7	Mean	> 7	4~7	Mean	> 7	4~7	Mean
	mg sprout^{-1}														
Cultivar (C)															
Eunhakong	58.1	63.6	59.8	25.0	23.2	24.5	1.8	1.7	1.7	84.9	88.4	86.0	26.8	24.8	26.2
Hannamkong	59.3	62.4	59.7	23.2	13.5	21.5	2.2	1.7	2.1	84.7	77.6	83.3	25.4	15.2	23.6
$LSD_{.05}$	ns	ns	ns	1.3	1.7	1.2	ns	ns	ns	ns	5.3	ns	ns	1.7	1.3
Light quality during 24-hour imbibition (L)															
Red	59.7	62.9	60.7	23.5	18.8	22.4	2.2	1.8	2.1	85.4	83.4	85.3	25.7	20.5	24.6
Fared	59.8	60.9	60.2	24.5	18.2	23.1	1.8	1.4	1.7	86.0	80.6	85.0	26.3	19.6	24.8
Blue	57.5	65.4	59.0	24.2	17.6	23.0	1.9	1.7	1.8	83.5	84.7	83.9	26.0	19.4	24.8
Dark (control)	57.9	62.7	59.1	24.3	18.8	23.4	2.0	1.8	2.0	84.2	83.2	84.5	26.3	20.5	25.3
$LSD_{.05}$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C × L	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

†After three time of drying for 0.5 hour at 25°C following 5.5 hour water imbibition, seeds were soaked in 4.0 ppm BA solution.
‡Hypocotyl length (cm) of soybean sprouts cultivated for 6 days after the above imbibition.

hypocotyl length and diameter, lateral root formation because all light qualities had the same component, total weights and economic yield (Table 3).

In conclusion, light qualities and illumination period done during seed imbibition for soybean sprout culture affected seed germination, sprout growth and morphology. Light treatments forced during 24-hour imbibition, however, did not induced any lateral roots but showed nearly the same growth and morphological characteristics.

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