Peer-reviewed version available at Agronomy 2018, 8, 207; doi:10.3390/agronomy8100207

Article

The Effects of Different Supplementary Light Sources on the Quality of Grafted Tomato seedlings and the Expression of Two Photosynthetic Genes

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Abstract: Supplementary lighting is commonly used in high-quality seedling production. In this study, grafted tomato seedlings were grown for 10 days in a glasshouse with a 16-hour daily supplementary lighting at 100 μmol·m^{-2}·s^{-1} PPFD from either high pressure sodium (HPS), metal halide (MH), far-red (FR), white LEDs (W), or mixed LEDs ($W_1R_2B_1$) to determine which light sources improve the seedling quality. The control did not have any supplementary light. The physiological parameters and the expression of genes related to photosynthesis were analyzed. The results show that the root length, biomass, number of leaves, chlorophyll (SPAD), dry weight to height ratio (WHR) of the scion, and specific leaf weight (SLW) were the greatest for grafted seedlings grown in $W_1R_2B_1$. The level of root ball formation was the greatest for seedlings grown in $W_1R_2B_1$, followed by those grown in W, HPS, and MH. Seedlings grown in FR did not fare very well, as they were very thin and weak. Moreover, the expression of two photosynthetic genes (*PsaA* and *PsbA)* was significantly increased by $W_1R_2B_1$ and W, which suggests that the plastid or nuclear genes might be regulated. The overall results suggest that $W_1R_2B_1$ was the most suitable light source to enhance the quality of grafted tomato seedlings. The results of this study could be used as a reference for seedling production in glasshouses, and provide new insights in the research of lights affecting the development of plants.

Keywords: WHR; SLW; *PsaA*; *PsbA*

1. Introduction

Light is an essential environmental factor affecting the growth and development of plants. It not only provides the basic energy to plants, but also plays a key role in their development [1]. Light quality refers to the composition of light with respect to the wavelengths that are effective in photosynthesis, which has a significant influence on plant morphology [2]. In winter and rainy periods of summer, natural light is often insufficient to induce the maximum growth and development of plug seedlings in greenhouses, which leads to a prolonged cultivation time and unexpected delays in delivery to growers in South Korea. Providing supplementary artificial lighting to plug seedlings may be a solution to this problem. Therefore, a study of the effects of the supplementary light quality on the

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development of seedlings can provide a practical guidance on the effective production of high quality seedlings.

Fluorescent lamps (FL), high-pressure sodium lamps (HPS), metal halide lamps (MH) and light emitting diodes (LEDs) are widely used in glasshouses to aid the production of grafted seedlings [3,4]. FL is the cheapest light source with a long working life (about 12000 hours) with a relatively low energy requirement, and is commonly used in plant tissue culture. However, FL contains a lot of green light, which often leads to an excessive growth of plants. HPS has a high luminous efficiency, high power, and a long working life (about 12,000 hours), and is consisted of a high level of red (R) light and low levels of blue (B) and green (G) lights. The standard sodium vapor pressure of HPS is about 10 kPa. Despite its operational benefits, because HPS is a thermal light source, the surface temperature is too high for plants [5]. MH is a discharge lamp filled with metal halides, mercury, and argon. The light emission is accomplished by the combination of electrons with ionized metal atoms [6]. Different spectra can be obtained by changing the composition of MH. The variability of spectrum and a high luminous efficiency makes MH one of the most commonly used light sources in glasshouses. However, damaged or neglected MH can pollute the environment with its mercury content. LEDs have recently been widely applied in plant production, as they carry the benefits of small size, long life, narrow spectral output, low mass and low energy consumption [7,8]. As a solid light source, LEDs do not pollute. Due to their advantages, LEDs are widely used in Holland, South Korea, Japan, the United States, and other countries, especially due to the successful research of high-power LEDs in recent years [9,10].

PsaA and *PsbA* respectively encode the core components of photosystem I (PSI) and photosystem II (PS II). The expression of *PsaA* and *PsbA* was regulated by plastid-encoded RNA polymerase, which was recruited by light signal [11]. The expression of *PsaA* and *PsbA* proteins can be enhanced by R and B lights [12]. Moreover, the synthesis and degradation of *PsbA* protein are light-regulated [13,14]. Most importantly, the deletion of *PsaA* and *PsbA* in tobacco (*Nicotiana tabacum* cv. Petit Havana) resulted in a simultaneous change of genes located in both the nucleus and the chloroplast [15]. Therefore, the light-dependent regulation of *PsaA* and *PsbA* can help explain the difference in growth and development of plants.

This study was carried out to determine the most effective supplementary light source in the quality improvement of two cultivars of tomato (*Solanum lycopersicum* L.), ‘Super Sunload’ and ‘Super Dotaerang,’ which were grafted onto the ‘B-Blocking’ tomato rootstocks. Seedlings were grown for 10 days in a glasshouse with an average daily maximum light intensity of 490 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD coming from the sun, with a 16-hour daily supplementary lighting at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD from either HPS, MH, FR, white LEDs (W), or mixed (W:R:B =1:2:1) LEDs ($W_1R_2B_1$). The control (CK) did not receive any supplementary lighting. The growth parameters and photosynthesis related genes of the seedlings were assessed to determine the effects of the different light sources on the grafted tomato seedlings.

2. Materials and Methods

2.1. Plant Materials

Commercially available grafted seedlings of two cultivars of tomato (*Solanum lycopersicum* L.) were obtained from Green Plug Seedling Company (Haman, Republic of Korea). ‘Super Sunload’ (Sakata Korea Co., Ltd., Seoul, Republic of Korea) and ‘Super Dotaerang’ (Koregon Co., Ltd., Anseong, Republic of Korea) seedlings were grafted onto ‘B-Blocking’ (Takii Korea Co., Ltd., Seoul, Republic of Korea) tomato rootstocks. Seedlings were all grown in 40-square cell plug trays containing a commercial medium (Super Mix, NongKyung Co., Jincheon, Republic of Korea). The two genotypes were selected as these two cultivars are extensively used for grafting in South Korea because of their high ratio of seed germination and fruit quality.

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2.2. Grafting Procedures

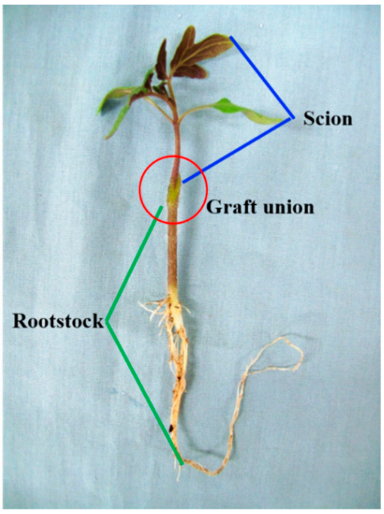

Figure 1. The grafting method used for tomato plug seedlings.

The cleft grafting method was used to graft the tomato seedlings (Figure 1). Rootstock seedlings were decapitated and a 1.0-1.5 cm long, 3/4 stem diameter deep longitudinal cut was made in a downward direction. The scion was pruned to have 1–3 true leaves and the lower stem was cut in a slant angle to make a tapered wedge. After placing the scion into the split made on the rootstock, a clip was placed to hold them in position until the graft union healed.

2.3. Light Treatments

The well-healed grafted seedlings were grown for 10 days in a glasshouse with an average daily maximum light intensity of 490 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD coming from the sun with a 16-hour daily supplementary lighting at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD from either HPS (HLF020600, BLV Licht Co., Ltd., Steinhöring, Germany), MH (SLMH-400, Sunlumen Co., Ltd., Gyeongju, Republic of Korea), FR (PAR38IR, Philips Lighting, Amsterdam, the Netherlands), white LEDs (W) (GOLED11/12, Victory Lighting Co., Ltd., Seoul, Republic of Korea), or mixed (W:R:B =1:2:1) LEDs ($W_1R_2B_1$) (Custom made, SungKwang LED Co., Ltd., Incheon, Republic of Korea) to check which light source most improves the seedling quality. The control (CK) did not have supplementary lighting. The spectral quality of each supplementary light treatment is shown in Figure 2. The cultivation environment had 30/25℃ day/night temperatures, 80±5% RH, and a 14-hour natural photoperiod. The spectral distributions were measured by a portable spectroradiometer (Spectra Light ILT 950, International Light Technologies, Inc., Peabody, MA, USA)

2.4. Data Collection and Analysis

After 10 days of supplementary light treatments, the length of the scion and root, scion diameter, chlorophyll level (SPAD), fresh and dry weights of the scion and root, number of leaves per seedling, leaf length and width, leaf thickness, specific leaf weight (SLW), and scion dry weight to height ratio (WHR) were measured for the grafted tomato seedlings.

A randomized complete block design with 3 replications and 9 seedlings in each replication was employed in this experiment. The treatment locations in a controlled environment were randomly laid out to minimize location effects. The collected data were analyzed for statistical significance with the SAS (Statistical Analysis System, V. 9.1, Cary, NC, USA) program. The experimental results were subjected to an analysis of variance (ANOVA) and the Duncan multiple range test.

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2.5. Quantitative real-time PCR Analysis

Total RNA was extracted from tomato leaves using the Easy-Spin total RNA extraction kit (iNtRON Biotechnology, Seoul, Republic of Korea) and was used for cDNA synthesis by GoScript Reverse Transcription System (Promega, Madison, WI, USA) according to the manufacturer's protocols. Gene expression was determined using the Rotor-Gene Q detection system (Qiagen, Hilden, Germany) with a software (version 2.3.1). Reactions were performed in a 20 μL total volume containing 1 μL of cDNA, 0.5 μL of each amplification primer (10 μM) (Supplementary Table 1), 10 μL of 2× AMPIGENE qPCR Green Mix Lo-ROX (Enzo Life Sciences Inc., Farmingdale, NY, USA), and 8 μL of ddH_2O. The relative expression of each gene was determined using the $2^{-\triangle\triangle Ct}$ method, and 18S was used as the reference gene.

3. Results

3.1. The Spectra of Different Supplementary Light Sources

As shown in Figure 2, HPS has a high irradiance between 570 and 620 nm as well as between 820 and 830 nm, which are respectively yellow/orange, and far-red, light. MH has a relatively scattered spectrum, with peak irradiances occurring for violet, blue, green, yellow, orange, red and far-red lights. FR has a high irradiance between 620 and 1050 nm, which are red and far-red lights. W is enriched in 420-475 nm and 500-650 nm, which are violet, blue, green, yellow, orange, and red lights. Lastly, $W_1R_2B_1$ contains relatively unitary lights of blue and red.

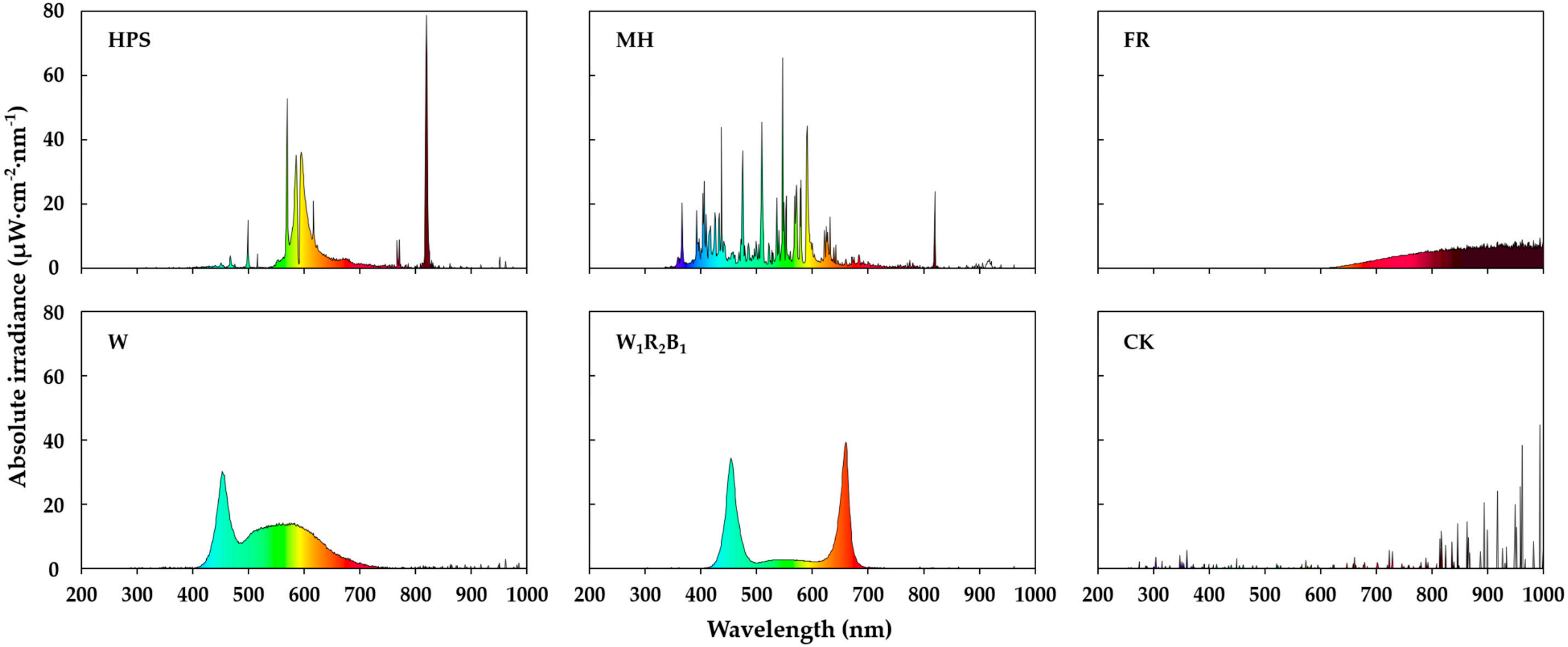

Figure 2. The spectral qualities of the different supplementary light sources (HPS, MH, FR, W, $W_1R_2B_1$ and CK) used in the experiment.

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3.2. The Growth and Development of Tomato Seedlings after the Supplementary Light Treatments in the Glasshouse

The results show that different light sources significantly affected the morphological characteristics of grafted tomato seedlings. The grafted seedlings of the two tomato cultivars grown in W had the greatest scion length, followed by seedlings grown in $W_1R_2B_1$, HPS and MH. Seedlings grown in FR and CK had the shortest scions. Seedlings in $W_1R_2B_1$ had the greatest scion diameter, followed by those in W, HPS, and MH. CK and FR produced very thin and weak seedlings. The chlorophyll level (SPAD) was the lowest for seedlings grown in FR. Seedlings grown in the two LED treatments, especially those in $W_1R_2B_1$, had long roots with high fresh weights. $W_1R_2B_1$ also produced seedlings with the highest number of leaves and the greatest leaf quality (leaf length, width and thickness) (Table. 1).

The seedling morphology of tomato 'Super Sunload' and 'Super Dotaerang' affected by 10-day light treatments are shown in Figure 3. It could be clearly observed that $W_1R_2B_1$, followed by W, were the most effective in promoting the level of root ball formation. The roots of seedlings grown in CK and FR were so weak that the seedlings could not be taken out together with the growing medium, as the roots could not wrap the medium in the plugs.

Table 1. The effects of the 10-day supplementary light treatments on the growth and development of grafted tomato seedlings.

Cultivar (C)	Light source (LS)	Scion		Leaf					Root	
		Length (cm)	Stem diameter (mm)	No.	Length (cm)	Width (cm)	Thickness (mm)	Chlorophyll (SPAD)	Length (cm)	Fresh weight (g)
'Super Sunload'	HPS	20.2 ab[z]	6.15 abc	10.3 ab	12.7 a	9.0 a	0.49 b	51.0 ab	12.6 a	0.95 ab
	MH	18.9 ab	5.84 abc	9.3 bc	13.3 a	8.6 ab	0.55 ab	45.5 bc	15.3 a	1.18 ab
	FR	15.5 b	5.21 c	7.7 d	10.9 bc	7.5 bc	0.47 b	41.0 c	12.5 a	0.73 b
	W	24.6 a	6.27 ab	11.0 a	13.5 a	8.5 ab	0.57 ab	57.0 a	14.7 a	1.37 a
	$W_1R_2B_1$	19.0 ab	6.55 a	11.0 a	12.6 ab	8.3 ab	0.68 a	56.8 a	15.7 a	1.41 a
	CK	16.1 b	5.33 bc	8.5 cd	9.7 c	6.9 c	0.45 b	38.9 c	8.1 b	0.71 b
'Super Dotaerang'	HPS	17.2 b	6.01 bc	9.8 ab	12.1 a	7.9 a	0.46 a	52.7 ab	13.4 ab	0.96 bc
	MH	15.6 bc	5.87 bc	8.8 bc	11.2 ab	7.6 a	0.47 a	44.9 bc	11.1 b	0.80 bc
	FR	12.3 c	4.69 c	7.7 c	9.7 b	5.7 b	0.47 a	41.0 c	6.6 c	0.53 c
	W	23.3 b	6.38 b	9.7 ab	11.6 ab	7.3 a	0.44 a	48.7 bc	11.9 ab	1.18 b
	$W_1R_2B_1$	17.5 a	7.86 a	11.2 a	12.3 a	7.8 a	0.58 a	61.0 a	14.8 a	1.79 a
	CK	12.6 c	5.60 bc	7.3 c	9.8 b	6.6 ab	0.51 a	43.1 c	7.6 c	0.57 c
F-test										
LS		***[y]	*	***	***	***	*	***	***	***
C		***	NS	*	***	***	*	NS	*	NS
LS*C		***	*	NS	NS	NS	**	*	***	*

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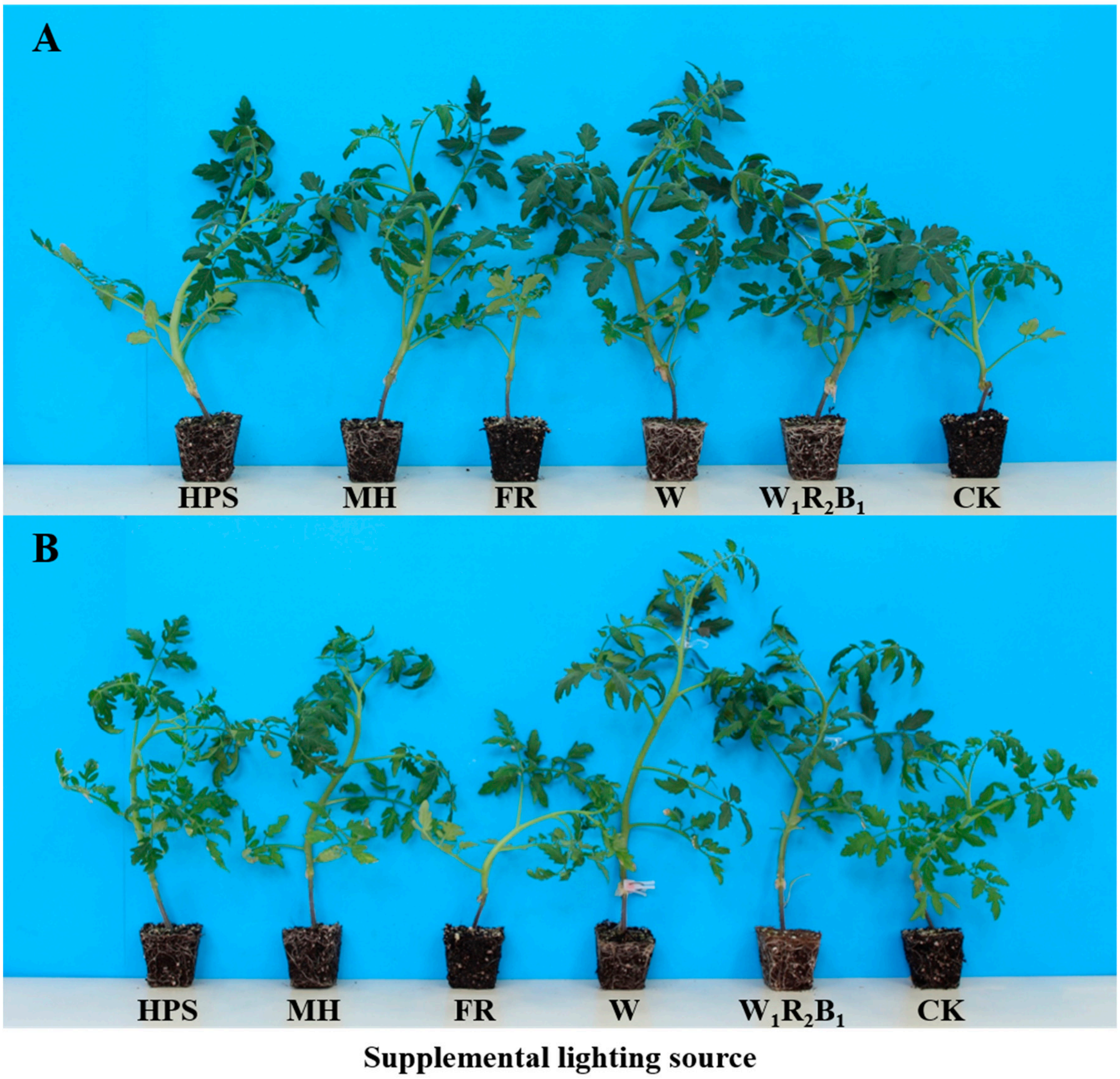

Figure 3. The effects of the 10-day supplementary light treatments on the morphology of grafted tomato seedlings 'Super Sunload' (A) and 'Super Dotaerang' (B).

3.3. Biomass of the Shoots and Roots

The dry weights of the shoots and roots are shown in Figure 4. The seedlings grown in $W_1R_2B_1$ had the greatest scion dry weights. There were no significant differences in the dry weights of seedlings grown in HPS, MH and W. Seedlings grown in FR and CK had very low scion dry weights. Seedlings grown in $W_1R_2B_1$ and W had the greatest root dry weights, followed by those grown in HPS and MH.

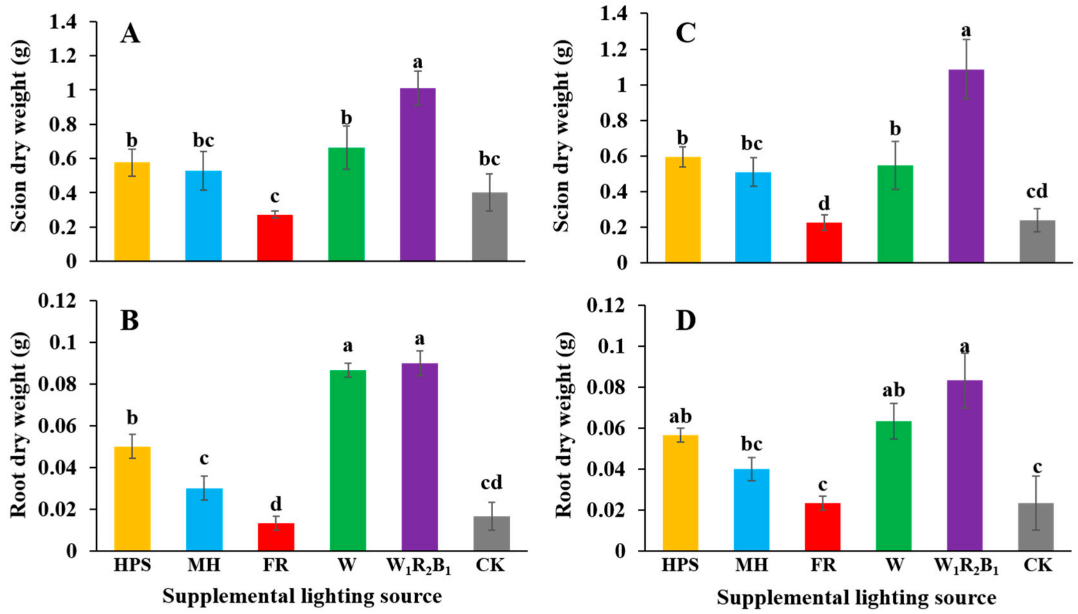

Figure 4. The effects of the supplementary lighting on the dry weights of the shoot and root of two tomato cultivars 'Super Sunload' (A, B) and 'Super Dotaerang' (C, D) grafted onto the 'B-Blocking' rootstocks. The error bars represent the SEs of nine biological replicates (n = 9). The significant differences among treatments are indicated by lower case letters at $p \leq 0.05$ according to the Duncan test.

3.4. Evaluation of the Seedling Quality

The qualitative objective of seedling production is to grow compact and sturdy seedlings. In order to assess the quality of the seedlings, the scion dry weight and scion height ratio (WHR) was calculated. The WHR of seedlings grown in $W_1R_2B_1$ was the greatest in this study (Figure 5 A, C). Seedlings grown in $W_1R_2B_1$ also had the greatest specific leaf weight (SLW) (Figure 5 B, D).

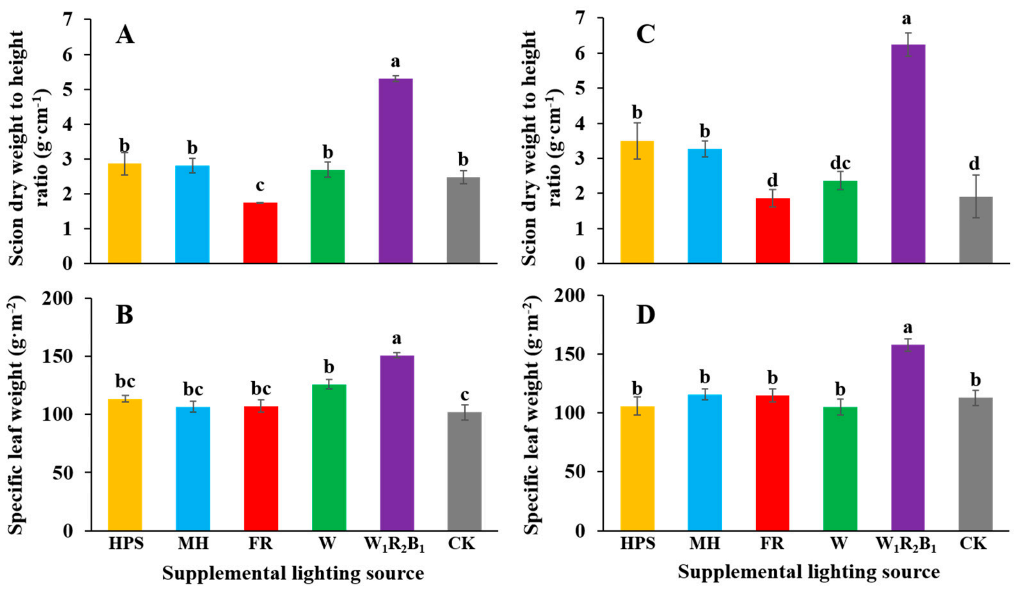

Figure 5. The effects of the supplementary lighting on the scion dry weight to height ratio and specific leaf weight of two tomato cultivars 'Super Sunload' (A, B) and 'Super Dotaerang' (C, D) grafted onto the 'B-Blocking' rootstocks. The error bars represent the SEs of nine biological replicates (n = 9). The significant differences among treatments are indicated by lower case letters at $p \leq 0.05$ according to the Duncan test.

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3.5. The Expression of Photosysthesis Related Genes

Genes encoding the PSI and PSII reaction center proteins were significantly affected by the different supplementary light sources (Figure 6). Generally, W and $W_1R_2B_1$ enhanced the expression of *PsaA* and *PsbA*, while FR had the opposite effects on the expression of the two genes. HPS had no significant influence on the expression of *PsaA* and *PsbA* when compared to CK. Interestingly, the relative expression of *PsaA* in 'Super Sunload' was 5.5- and 8.4-fold, while that of *PsbA* was 10.9- and 3.1-fold under W and $W_1R_2B_1$, respectively,. It was also observed in 'Super Dotaerang' that a dramatic increase of the expression of one gene resulted in a relatively lower increase in the expression of the other gene.

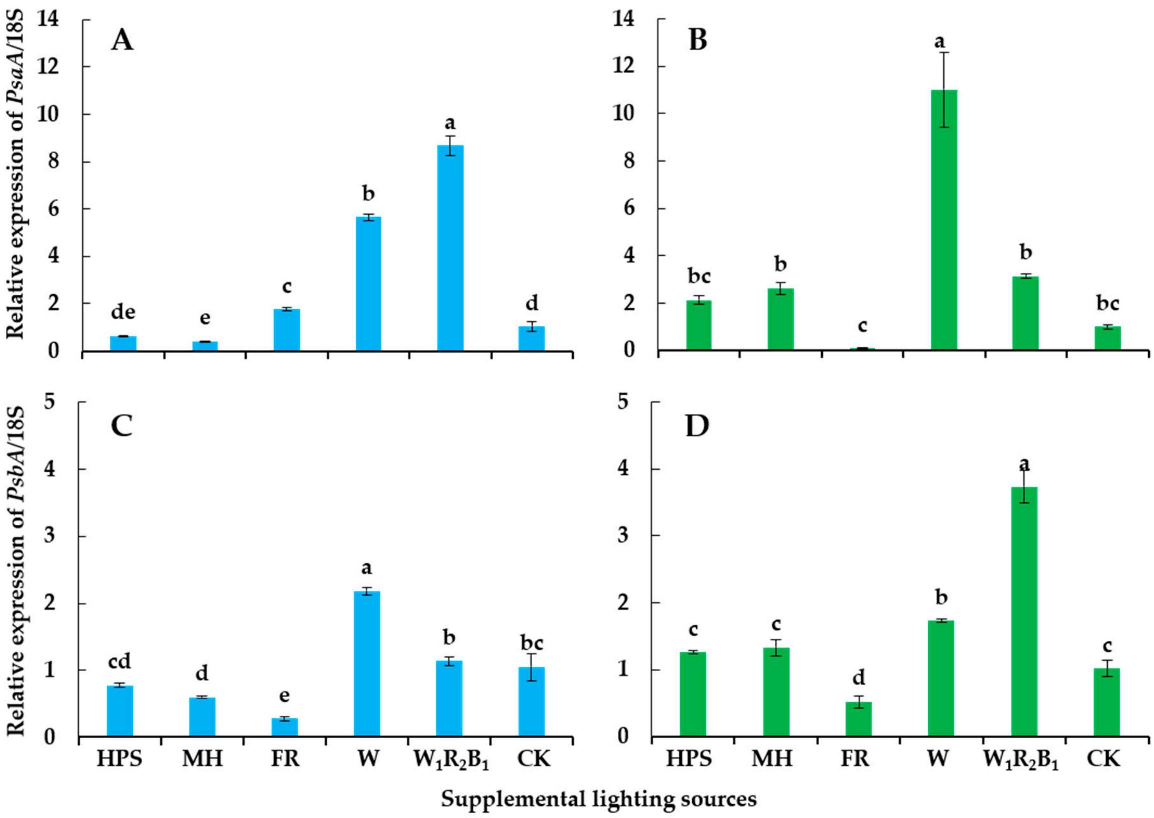

Figure 6. The relative expressions of *PsaA* and *PsbA* in two tomato cultivars 'Super Sunload' (A, C) and 'Super Dotaerang' (B, D) grafted onto the 'B-Blocking' rootstocks. The bar diagrams among the different treatments represent the mean ± SEs of three technological replicates (n = 3). Significant differences among treatments are indicated by lower case letters at $p < 0.05$ according to the Duncan' multiple range test.

4. Discussion

The chloroplast mainly absorbs R and B lights for photosynthesis [16]. Our study considered the spectra of five commonly used light sources, and found that W and $W_1R_2B_1$ had abundant R and B lights. Moreover, grafted tomato seedlings developed well under W and $W_1R_2B_1$. Previous studies have shown that R light inhibits internode elongation and delayed flower differentiation [17], and works on the accumulation of anthocyanins, chlorophyll and carotenoids [18-20]. R light helps plants resist biological and abiotic stresses [21]. In some cases, FR can counteract the effects of R light [22]. Supplementary FR light can promote plant growth, which may be due to the increase in the leaf area and the subsequent increase in light absorption [23]. For *Arabidopsis thaliana*, plants grown in a lower R/FR condition had bigger leaves, higher biomass, and stronger resistance to low temperatures than those grown in a higher R/FR condition [24]. B light suppressed hypocotyl elongation and promoted cotyledon expansion in *Arabidopsis thaliana*. R light, on the other hand, promoted both the hypocotyl elongation and cotyledon expansion [25]. Furthermore, R light could

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help increase the plant biomass [26], while B light could inhibit lateral shoot growth and internode elongation to prevent excessive growth [27]. Therefore, the good development of tomato seedlings in W and $W_1R_2B_1$ may be the result of sufficient R and B lights.

Numerous studies have lately shown that providing plants with single R or B light is insufficient to promote their photosynthesis and growth. Indeed, most studies suggest that combining B and R lights are the most effective in promoting the growth and development of plants. *Lactuca sativa*as had higher yields when grown under a combination of R and B lights than when grown under R light [28]. The studies on *Chrysanthemum morifolium* showed that a combination of R and B lights increased the net photosynthetic rate. The combination of R and B lights could maximize the dry weight and leaf area. On the contrary, the combination of B, R and FR lights delayed the growth of chrysanthemum [29,30]. Jang et al. found that the mixture of B and R lights was the most beneficial for healing and growing grafted pepper seedlings [31]. In our previous study of grafted tomato seedlings treated with a combination of R and B lights and $W_1R_2B_1$ (data not shown), it was demonstrated that $W_1R_2B_1$ was the most suitable for developing the vascular bundles and stomotal behaviors [32]. It was speculated that W light contained lights of different wavelengths that provided plants with various slight, but essential, lights.

Growers prefer stocky seedlings as they are easy to transport and have a high survival rate after transplanting. Our results showed that $W_1R_2B_1$ best promotes the shoot and root biomass. Moreover, seedlings grown in $W_1R_2B_1$ had the highest WHR as they had more biomass and were shorter.

PsaA and *PsbA* are chloroplast genes encoding the P700 apoproteins of PSI and the D1 protein of PSII, respectively. The expression of *PsaA* and *PsbA* can regulate the plastid-encoded genes [33] as well as the nuclear genes [15]. Plastid encoded polymerase and nuclear encoded polymerase are interactional [11,15,34,35]. Therefore, the quality improvement of tomato seedlings under $W_1R_2B_1$ may be due to the up-regulation of *PsaA* and *PsbA*. However, the reason for the different extents of up-regulation is yet unknown.

5. Conclusions

This study demonstrated that the root length, biomass, number of leaves, chlorophyll level (SPAD), scion dry weight to height ratio (WHR), and specific leaf weight (SLW) were the greatest for seedlings grown in $W_1R_2B_1$ among all considered treatments. The level of root ball formation was the greatest for seedlings grown in $W_1R_2B_1$, followed by those in W, HPS, and MH. Seedlings grown in FR were very thin and weak. The light spectra of W and $W_1R_2B_1$ environments contained abundant R and B lights, which were largely absorbed and utilized by plants. The expression of *PsaA* and *PsbA* indicated that their up-regulation might contribute to the improvement in the quality of tomato seedlings. Overall, the results suggest that $W_1R_2B_1$ is the most suitable light source for promoting the quality of grafted tomato seedlings.

Author Contributions: Conceptualization, BR.J.; Methodology, BR.J and H.W.; Formal Analysis, J.H., C.L., J.Z. and M.W.; Resources, BR.J.; Data Curation, H.W.; Writing-Original Draft Preparation, H.W.; Writing-Review & Editing, BR.J.; Project Administration, BR.J.; Funding Acquisition, BR.J, H.W, J.H, C.L, M. W. and DI.K.

Funding: This research was funded by Korea Rural Development Administration, Project No. PJ012773022018. H. W., J. H., C. L., and M. W. were supported by a scholarship from the BK21 Plus Program, Ministry of Education, Republic of Korea.

Acknowledgements: This study was carried out with a support from the Korea Rural Development Administration (Project No. PJ01277302). Hao Wei, Jiangtao Hu, Chen Liu, Mengzhao Wang, Jin Zhao and Dongil Kang were supported by a scholarship from the BK21 Plus Program, Ministry of Education, Republic of Korea. HPS was provided by USHIO Korea, Inc.

Conflicts of Interest: The authors declare no conflict of interest.

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