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# Effect of Different Combinations of Red and Blue LED Light on Growth Characteristics and Pigment Content of In Vitro Tomato Plantlets

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**Abstract:** The aim of this study was to evaluate the growth characteristics and pigment content of tomato plantlets grown under various ratios of red (R) (661 nm) and blue (B) (449 nm) LED light. In this study, three different ratios of R and B (RB) light such as 5:01, 10:01, and 19:01 along with R (100%) were used. The photosynthetic photon flux density (PPFD), and photoperiod of the growth chamber was 120 ± 5 μmol $m^{-2}s^{-1}$ and 16/8 h (day/night), respectively. Tomato plantlets were cultured for six weeks in the growth chamber. It was shown that tomato plantlets had higher photosynthesis rate, higher pigments content, higher growth characteristics (e.g., number of leaves, leaf area, shoot number, root number, root length, dry, and fresh mass), and greater surviving rate under the R:B = 10:01 ratio among the treatments. The plantlets showed at least a threefold decrease in photosynthesis rate, as well as a significant abnormal stem elongation when grown under 100% R light. It is concluded that the RB ratio of 10:01 showed excellent performance in all growth parameters. This result has shown that the optimum lighting environment improves tomato plantlet cultures in vitro.

**Keywords:** light-emitting diode (LED); tomato plantlets; biomass; photosynthesis; pigments

## 1. Introduction

In vitro plant culture is a valued method in achieving improved disease free identical plant seedlings. It has enormous roles in the propagation of plants in large quantities with desired characters [1]. Light is the most vital element to regulate in vitro plant growth and development.

Artificial light should provide photons in the spectral region that is involved in photosynthesis and in the photomorphogenic responses of plant culture in vitro [2,3]. Light acts as a signaling mechanism through different light receptors and provides the required energy for plant growth and development [4]. The development of in vitro plantlets can be improved by tuning the light quality, quantity, and photoperiod in the growth environment [5].

Artificial light emitting diode (LED) light has been strategically used for the growth of many plant species including chrysanthemum [5], cabbage [6], cymbidium [7], rapeseed [8], and strawberry [9]. Among the different LED light qualities, blue (B) (420–450 nm) and red (R) (600–700) are the most effective light spectra for the determination of plant growth and development [10]. It was previously reported that the absorption percentage of B and R light is about 90% among the light

spectra [11]. In addition, monochromatic B and R light alone could not meet the requirements of plant growth and development [12]. The absence of one of the two light wavebands (B or R) creates photosynthetic inefficiencies [13]. B light is predominantly absorbed by chlorophyll (*chl*) *a* and *chl b*, which assimilates the photosynthetic $CO_2$ thus initiating the photosynthetic process. In contrast, R light produces a narrow-spectrum light that regulates photomorphogenesis, energy distribution, and the photosynthetic apparatus [4]. It is noted that artificial R light expressively enhances in vitro stem elongation of potato and *Pelargonium* plantlets [14,15]. Nhut et al. [9] demonstrated that growth characteristics of strawberry increased when grown under 70% R and 30% B light.

Tomato (*Solanum lycopersicum* L.) is the second most crucial vegetable crop in the world after potato [16]. It is grown in almost every country and achieved tremendous popularity due to its high nutritional value [17]. Tomato seedling production by tissue culturing is an imperative approach to improve the seedling quality [18].

Moreover, cultivated tomatoes suffer from many diseases that are caused by bacteria, fungi, viruses, and nematodes [19,20]. It is reported that tomato plant grown under different combinations of LED light has a confrontation to the various diseases and pathogen attack [21]. Plenty of research has already been done on tomato in vitro culture [22–24]. However, information on the effect of a combination of artificial R and B LED light application on in vitro tomato plantlets is still inadequate so far. It is assumed that an efficient combination of RB light would improve the tomato plantlets quality.

## 2. Materials and Methods

### 2.1. Plant Materials and Culture Conditions

Tomato (*Solanum lycopersicum* L.) seeds was sterilized in 8% Clorox solution (sodium hypochlorite) for 10 min followed by three times rinsing with distilled water. The nutrient agar medium was prepared according to Murashige and Skoog [25] with slight modification (MS + IAA 0.2 mg/L + BAP 0.05 mg/L, $\frac{1}{2}$ $NH_4NO_3$, $\frac{1}{2}$ $KNO_3$) at 26/22 °C (light/dark) temperatures adjusting pH at 5.8 and inoculated by autoclaving (121 °C for 20 min).

Tomato explant was grown in a growth chamber equipped with fluorescent lamp (E15, Conviron, Winnipeg, Canada) under photosynthetic photon flux density (PPFD) of 60 ± 5 µmol $m^{-2}s^{-1}$, 16/8 light/dark regime, 23 ± 1 °C temperature. After three weeks, tomato explants were excised (1–2 cm hypocotyl segments) and cultured in the same nutrient medium (described above) supplemented with 30 g sucrose and 2 mg/L of BAP in order to grow tomato plantlets.

### 2.2. Plantlet Culture and LED Light Treatments

Tomato plantlets were cultured under different artificial red (R) (661 nm) and blue (B) (449 nm) light treatment such as R:B = 5:01; 10:01, 19:01, and R 100% in a growth chamber (General Electric Lighting Solutions, Lachine, Quebec, Canada). The photosynthetic photon flux density (PPFD) of 120 ± 5 µmol $m^{-2}s^{-1}$, photoperiod of 16/8 h (light/dark), and temperature of 23 ± 1 °C was maintained in the growth chamber. The above environmental parameters of the growth chamber were fixed based on a previous study for the tomato plantlet cultures in vitro (data not published).

### 2.3. Evaluation of Plantlet Growth and Pigment Content

The tomato plantlets were cultured for six weeks and growth characteristics were analyzed by measuring the stem height, number of leaves, leaf area, shoot number, root number, root length, fresh, and dry weight. Leaf area was determined by taking a digital image of leaves and using Image J software (Bethesda, Maryland, USA). The plantlet fresh mass (FM) was assessed immediately after harvesting, and dry mass (DM) was determined after drying in an oven at 65 °C for 24 h. Chlorophyll pigments were analyzed according to method described by Lichtenthaler [26] using a spectrophotometer (PowerWave™ XS Microplate Reader, BioTek Instruments, Inc. Vermont, USA).

The content of *Chl a*, *Chl b*, total *Chl* and carotenoid were calculated based on plant fresh weight. Five tomato plantlets from each treatment were selected for the analysis of growth and pigment content.

### *2.4. Photosynthesis Measurement*

The photosynthesis of the tomato plantlets was measured using an LI-6400 XT portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA) on the day of harvesting. The cuvette climate of the photosynthesis system was as follows: $CO_2$ 400 µmol $mol^{-1}$, airflow 200 ml $min^{-1}$, temperature 20 ± 1 °C, vapor pressure deficit (VPD) 10 ± 3 Pa, and RH 50 ± 10%. Three plantlets from each treatment were designated for the photosynthesis analysis. The wider leaves of tomato plantlets were subjected to measurement for photosynthesis.

### *2.5. Statistical Analysis*

The results were expressed as mean values and their standard errors (SE) using MS Excel software. Least significant differences among the light treatments were evaluated by the Tukey's HSD tests ($p < 0.05$). The experiment was repeated twice maintaining the same environment.

## 3. Results and Discussion

### *3.1. Plant Growth, Biomass, and Pigment Analysis*

The morphological features of tomato plantlets are shown in Figure 1. It was observed that healthy and vigorous tomato plantlets were attained when grown under the RB ratio of 10:01 among the light treatments. Likewise, the survival rate of tomato plantlets was higher when cultured under RB light than those under 100% R light. The highest survival rate (80%) of the tomato plantlets was attained under an RB ratio of 10:1 compared to 100% R light (60%) (Figure 2).

The fraction of B light in the RB combination had significant influence on growth characteristics and biomass of tomato plantlets (Table 1; Figure 3). It was observed that tomato plantlets had higher leaf number, leaf area, shoot number, root number, and root length in the combination of RB treatment compared to 100% R. In addition, the RB ratio of 10:01 showed the best performance considering growth characteristics and biomass production (fresh and dry mass) of tomato plantlets.

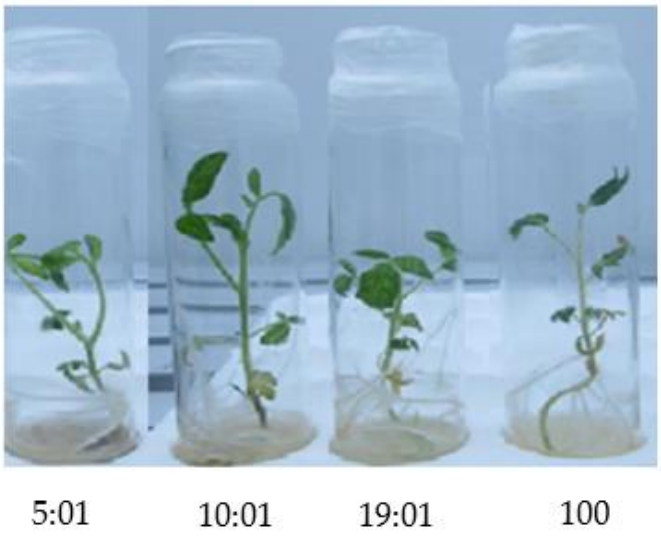



**Figure 1.** Tomato plantlets grown under different ratios of red (R) to blue (B) LED light. Photograph showing six weeks cultured plantlets.

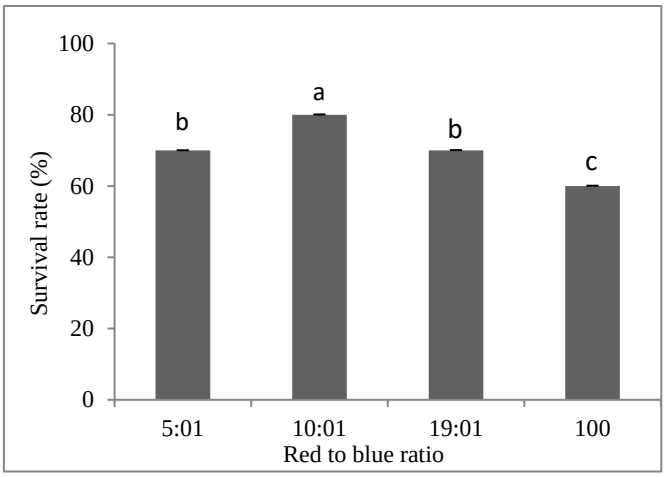



**Figure 2.** Surviving rate of tomato plantlets under different ratios of R to B LED light. Mean separation within columns by Tukey's HSD tests ($n$ = 5). Values labeled with different letters in a column are significantly different ($p < 0.05$).

However, the stem length was remarkably increased under 100% R light compared to other treatments (Table 1). The stem length of the tomato plantlet was 1.2, 1.0, and 1.5 times shorter under the RB ratio of 5:01, 10:01, and 19:01, respectively, compared to the 100% R light.

**Table 1.** Effects of various red-blue (RB) light ratios on the growth of tomato plantlets.

| LED Light Ratio | Stem Length (cm) | Leaf Number | Leaf Area ($cm^2$) | Shoot Number | Root Number | Root Length (cm) |
|---|---|---|---|---|---|---|
| RB 5:01 | $9.3^{b}$ | $5.5^{a}$ | $14.3^{a}$ | $2.1^{b}$ | $3.0^{a}$ | $2.1^{a}$ |
| RB 10:01 | $10.1^{a}$ | $5.9^{a}$ | $14.2^{a}$ | $2.8^{a}$ | $3.3^{a}$ | $2.4^{a}$ |
| RB 19:01 | $7.8^{b}$ | $5.1^{a}$ | $12.9^{b}$ | $2.5^{a}$ | $3.2^{a}$ | $2.2^{a}$ |
| R 100 | $12.1^{a}$ | $4.5^{b}$ | $13.3^{b}$ | $1.3^{c}$ | $2.6^{b}$ | $1.8^{b}$ |

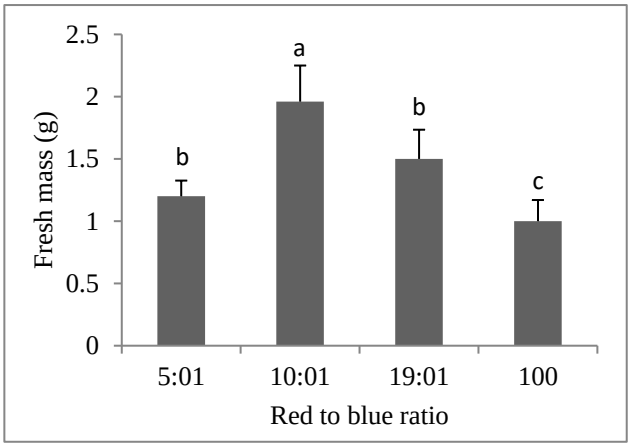
Mean separation within columns by Tukey's HSD tests ($n$ = 5). Values labeled with different letters in a column are significantly different ($p < 0.05$).

The results of the analysis of total chlorophyll, *chl a*, *chl b*, and carotenoids are presented in Table 2. It was observed that the *chl a* (565.02 μg/g), *chl b* (241.76 μg/g), total chlorophyll (806.79 μg/g), and carotenoids (190.36 μg/g) of tomato plantlets were significantly higher in RB light compared to 100% R light (412.71 μg/g, 181.66 μg/g, 594.36 μg/g, and 137.12 μg/g, respectively). The highest content of pigments was achieved at the RB ratio of 10:01 among the light combinations.

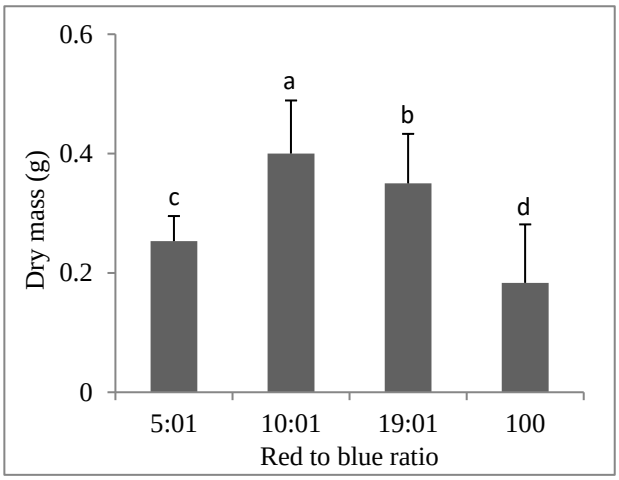
**Table 2.** Effects of various RB light ratios on the pigment contents of tomato plantlets.

| LED Light Ratio | Chlorophyll a (μg/g FW) | Chlorophyll a (μg/g FW) | Total Chlorophyll (μg/g FW) | Carotenoid (μg/g FW) |
|---|---|---|---|---|
| RB 5:01 | $443.39 \pm 12.47^{c}$ | $223.16 \pm 5.51^{ab}$ | $667.61 \pm 11.44^{c}$ | $182.91 \pm 3.19^{a}$ |
| RB 10:01 | $565.02 \pm 7.25^{a}$ | $241.76 \pm 8.68^{a}$ | $806.79 \pm 7.31^{a}$ | $190.36 \pm 7.48^{a}$ |
| RB 19:01 | $497.51 \pm 7.23^{b}$ | $212.66 \pm 9.12^{b}$ | $710.16 \pm 6.69^{b}$ | $172.14 \pm 3.56^{a}$ |
| R 100 | $412.71 \pm 12.73^{d}$ | $181.66 \pm 13.82^{c}$ | $594.36 \pm 16.91^{d}$ | $137.12 \pm 8.13^{b}$ |

Mean separation within columns by Tukey's HSD tests ($n$ = 5). Values labeled with different letters in a column are significantly different ($p < 0.05$).




**(A)**




**(B)**

**Figure 3.** Fresh (**A**) and dry (**B**) mass of tomato plantlets under different ratios of red to blue LED light. Mean separation within columns by Tukey's HSD tests ($n = 5$). Values labeled with different letters in a column are significantly different ($p < 0.05$).

The fraction of B light in the RB combination showed a great influence on the growth characteristics of tomato plantlets. It was reported that monochromatic light is not sufficient for the normal growth and development of plants [27,28]. Plants cannot have functioning physiological and morphological process without the combination of R and B light [29]. The potentiality of R and B light on plant growth and development has been intensively studied [30,31].

Our investigations revealed that tomato plantlets were physically healthy when cultured under various RB ratios and the highest growth was under the RB ratio of 10:01. In the absence of B light, the plantlets were abnormal (elongated). Excessive elongation of plantlets reduced the survival rate and hindered the process of transplanting and moving from one tray to another [32].

It was reported that the numbers of leaves of strawberry were higher when grown under RB light than when grow under 100% R light [9]. It was reported that R light affected stem elongation and decreased pigments content [33,34]. Our results are also in agreement with Appelgren [14] for Pelargonium, in which blue light strongly inhibited stem elongation.

It was observed that the biomass and leaf growth of tomato plantlets were significantly increased under RB light treatments as compared with monochromic R light treatments. This may be due to the maximum photosynthetic efficiency of plants grown under RB light, since this light wavelength range closely coincide with the absorption peaks of chlorophylls [35].

In the current study, RB light significantly increased chlorophyll and carotenoid content compared to 100% R. It was recognized that plant pigments have specific light absorption spectra [36]. For instance, *chl* and carotenoid have high light absorption at 400–500 nm (B light) and at 630–680 nm (R light), respectively [36]. The dose of RB light was plant species dependent; for instance, the appropriate RB dose was 1:1, 4:1, and 3:7 for the growth of lilium, banana, and strawberry, respectively [37–39]. The combination of RB light promoted the growth of kale, capsicum, and pepper plantlets, but the ideal proportion of RB light seemed to be plant species dependent [12].

B light was abundantly absorbed by photosynthetic pigments and a vital catalyst to increase the chlorophyll and carotenoid [6]. Results from the current study showed that tomato plantlets have high total chlorophyll and carotenoid pigments under RB ratio, which is consistent with the result of brassica plantlets in vitro [8]. Ma et al. [40] illustrated that key gene activity of the enzyme in chlorophyll and carotenoid pigments were stimulated by monochromatic B light resulting in higher pigments accumulation.

### 3.2. Photosynthesis Analysis of Tomato Plantlets

The tomato plantlets grown under monochromatic R light had reduced photosynthesis rate compared to different RB light treatments (Figure 4). The highest photosynthesis rate of the tomato plantlets was observed at the RB = 10:01 ratio. The photosynthesis rate of the tomato plantlets was 1.8, 2.6, and 1.6 times higher under RB ratio of 5:01, 10:01, and 19:01, respectively, compared to 100% R light.

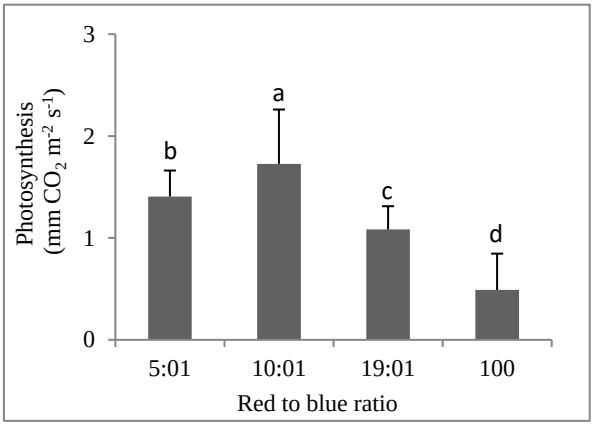



**Figure 4.** Photosynthesis of tomato plantlets under different ratios of red to blue led light. Mean separation within columns by Tukey's HSD tests ($n$ = 5). Values labeled with different letters in a column are significantly different ($p < 0.05$).

The combination of RB light has been proved to be effective in driving photosynthesis [29]. The combination of R and B light had the most photosynthetically effective wavebands. The absence of one of the two lights created photosynthetic inefficiencies for cucumber [13]. Our results indicated that photosynthesis of tomato plantlets efficiently improved by increasing the B light fraction in the RB light combination. The same trend of increasing photosynthesis was also observed in rice and cucumber [30,41]. This result is supported by analyzing the chlorophyll pigment. B light increased the chlorophyll molecule that makes photosynthesis possible [42]. A higher content of chlorophyll improved the efficiency of light absorption, which directly enhances the photosynthesis process [42]. Good quality tomato seedlings should be compact, with short internodes and firm stems, and large and intensive green leaves. Such seedlings guarantee optimal development of the root system after transplanting and have an effect on the quality and quantity of the plant yield [43].

## 4. Conclusions

Our result suggested that the fraction of B light in the RB combination has a crucial impact on optimal growth and development of tomato plantlets in vitro. The higher and lower proportion of B light in the RB combination impede the growth and developmental process. The optimal RB ratio was 10:01 for tomato plantlet cultures in vitro. This findings would be helpful for the design of artificial light for other plant species.

**Author Contributions:** M.T.N. designed and conducted the experiment, analyzed the data, and drafted the manuscript; M.O.K.A. and C.H.P. edited and revised the manuscript; M.L. supervised the research.

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

## References

1. Chen, J.; Henny, R.J.; McConnell, D.B. Development of new foliage plant cultivars. In *Trends in New Crops and New Uses*; ASHS Press: Alexandria, VA, USA, 2002; pp. 466–472.
2. Bula, R.J.; Morrow, R.C.; Tibbitts, T.W.; Barta, D.J.; Ignatius, R.W.; Martin, T.S. Light-emitting diodes as a radiation source for plants. *HortScience* **1991**, *26*, 203–205. [CrossRef] [PubMed]
3. Seabrook, J.E.A. Light effects on the growth and morphogenesis of potato (*Solanum tuberosum*) in vitro: A review. *Am. J. Potato Res.* **2005**, *82*, 353–367. [CrossRef]
4. Ouzounis, T.; Rosenqvist, E.; Ottosen, C.O. Spectral effects of artificial light on plant physiology and secondary metabolism: A review. *HortScience* **2015**, *50*, 1128–1135. [CrossRef]
5. Kurilcik, A.; Miklusytė-Canova, R.; Dapkuniene, S.; Zilinskaite, S.; Kurilcik, G.; Tamulaitis, G.; Duchovskis, P.; Zukauskas, A. In vitro culture of *Chrysanthemum* plantlets using light-emitting diodes. *Cent. Eur. J. Biol.* **2008**, *3*, 161–167. [CrossRef]
6. Li, H.; Tang, C.; Xu, Z.; Liu, X.; Han, X. Effect of different light sources on the growth of non-heading Chinese cabbage (*Brassica campestris* L.). *J. Agric. Sci.* **2012**, *4*, 262–273. [CrossRef]
7. Tanaka, M.; Takamura, T.; Watanabe, H.; Endo, M.; Yanagi, T.; Okamoto, K. In vitro growth of *Cymbidium* plantlets cultured under super bright red and blue light-emitting diodes (LEDs). *J. Hortic. Sci. Biotechnol.* **1998**, *73*, 39–44. [CrossRef]
8. Li, H.; Tang, C.; Xu, Z. The effect of different light qualities on rape seed (*Bassica napus* L.) plantlet growth and morphogenesis in vitro. *Sci. Hortic.* **2013**, *150*, 117–124. [CrossRef]
9. Nhut, D.T.; Takamura, T.; Watanabe, H.; Okamoto, K.; Tanaka, M. Responses of strawberry plantlets cultured in vitro under super bright red and blue light emitting diodes (LEDs). *Plant Cell Tissue Organ Cult.* **2003**, *73*, 43–52. [CrossRef]
10. Chen, X.L.; Yang, Q.C.; Song, W.P.; Wang, L.C.; Go, W.Z. Growth and nutritional properties of lettuce affected by different alternating intervals of red and blue LED irradiation. *Sci. Hortic.* **2013**, *223*, 4–52. [CrossRef]
11. Terashima, I.; Fujita, T.; Inoue, T.; Chow, W.S.; Oguchi, R. Green light drives leaf photosynthesis more efficiently than red light in strong white light: Revisiting the enigmatic question of why leaves are green. *J. Plant Cell Physiol.* **2009**, *50*, 684–697. [CrossRef]
12. Naznin, M.T.; Lefsrud, M.; Gravel, V.; Azad, M.O.K. Blue light added with Red LEDs enhance growth characteristics, pigments content, and antioxidant capacity in lettuce, spinach, kale, basil, and sweet pepper in a controlled environment. *Plants* **2019**, *8*, 93. [CrossRef] [PubMed]
13. Hogewoning, S.W.; Douwstra, P.; Trouwborst, G.; Leperen, W.V.; Harbinson, J. An artificial solar spectrum substantially alters plant development compared with usual climate room irradiance spectra. *J. Exp. Bot.* **2010**, *61*, 1267–1276. [CrossRef] [PubMed]
14. Appelgren, M. Effects of light quality in stem elongation of *Pelargonium* in vitro. *Sci. Hortic.* **1991**, *45*, 345–351. [CrossRef]

15. Aksenova, N.P.; Konstantinova, T.N.; Sergeeva, L.I.; Machackova, I.; Golyanovskaya, S.A. Morphogenesis of potato plants in vitro. I. Effect of light quality and hormones. *J. Plant Growth Regul.* **1994**, *13*, 143–146. [CrossRef]
16. Foolad, M.R. Redent advances in genetics of salt tolerance in tomato. *Plant Cell Tissue Organ Cult.* **2004**, *76*, 101–119. [CrossRef]
17. Bhatia, P.; Ashwath, N.; Senaratna, T.; Midmore, D. Tissue culture studies of tomato (*Lycopersicon esculentum*). *Plant Cell Tissue Organ Cult.* **2004**, *78*, 1–21. [CrossRef]
18. Evans, D.A. Somaclonal variation—Genetic basis and breeding applications. *Trends Genet.* **1989**, *5*, 46–50. [CrossRef]
19. Murphy, J.F.; Zehnder, G.W.; Schuster, D.J.; Sikora, E.J.; Polston, J.E.; Kloepper, J.W. Plant growth-promoting rhizobacterial mediated protection in tomato against *Tomato mottle virus*. *Plant Dis.* **2000**, *84*, 779–784. [CrossRef] [PubMed]
20. Sabrol, H.; Satish, K. Tomato plant disease classification in digital images using classification tree. In Proceedings of the 2016 International Conference on Communication and Signal Processing (ICCSP), Melmaruvathur, India, 6–8 April 2016; pp. 1242–1246.
21. Hui, X.U.; FU, Y.N.; LI, T.L.; Wang, R. Effects of different LED light wavelengths on the resistance of tomato against Botrytis cinerea and the corresponding physiological mechanisms. *J. Integr. Agric.* **2017**, *16*, 106–114.
22. Koeda, S.; Takisawa, R.; Nabeshima, T.; Tanaka, Y.; Kitajima, A. Production of Tomato yellow leaf curl virus-free parthenocarpic tomato plants by leaf primordia-free shoot apical meristem culture combined with in vitro grafting. *Hortic. J.* **2015**, *84*, 327–333. [CrossRef]
23. Sohrabi, F.; Nooryazdan, H.; Gharati, B.; Saeidi, Z. Evaluation of ten tomato cultivars for resistance against tomato leaf miner, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) under field infestation conditions. *Entomol. Gen.* **2016**, *36*, 163–175. [CrossRef]
24. Barari, H. Biocontrol of tomato Fusarium wilt by Trichoderma species under in vitro and in vivo conditions. *Cercet. Agron. Mold.* **2016**, *49*, 91–98. [CrossRef]
25. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
26. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *603*, 591–592. [CrossRef]
27. Kang, W.H.; Park, J.S.; Park, K.S.; Son, J.E. Leaf photosynthetic rate, growth, and morphology of lettuce under different fractions of red, blue, and green light from light-emitting diodes (LEDs). *Hortic. Environ. Biotechnol.* **2016**, *57*, 573–579. [CrossRef]
28. Darko, E.; Heydarizadeh, P.; Schoefs, B.; Sabzalian, M.R. Photosynthesis under artificial light: The shift in primary and secondary metabolism. *Philos. Trans. R. Soc. B* **2014**, *369*. [CrossRef] [PubMed]
29. Son, K.H.; Oh, M.M. Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. *HortScience* **2013**, *48*, 988–995. [CrossRef]
30. Matsuda, R.; Ohashi-Kaneko, K.; Fujiwar, K.; Goto, E.; Kurata, K. Photosynthetic characteristics of rice leaves grown under red light with or without supplemental blue light. *Plant Cell Physiol.* **2004**, *45*, 1870–1874. [CrossRef]
31. Ouzounis, T.; Frette, X.; Rosenqvist, E.; Ottosen, C.O. Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums, and campanulas. *J. Plant Physiol.* **2014**, *171*, 1491–1499. [CrossRef]
32. Garner, L.C.; Björkman, T. Mechanical conditioning of tomato seedlings improves transplant quality without deleterious effects on field performance. *HortScience* **1999**, *34*, 848–851. [CrossRef]
33. Hoenecke, M.E.; Bula, R.J.; Tibbitts, T.W. Importance of 'blue' photon levels for lettuce seedlings grow under red light-emitting diodes. *HortScience* **1992**, *27*, 427–430. [CrossRef] [PubMed]
34. Tripathy, B.C.; Brown, C.S. Root-shoot interaction in the greening of wheat seedlings grown under red light. *Plant Physiol.* **1995**, *107*, 407–411. [CrossRef] [PubMed]
35. Shin, K.S.; Murthy, H.N.; Heo, J.W.; Hahn, E.J.; Paek, K.Y. The effect of light quality on the growth and development of in vitro cultured Doritaenopsis plants. *Acta Physiol. Plant.* **2008**, *30*, 339–343. [CrossRef]
36. Lian, M.L.; Murthy, H.N.; Paek, K.Y. Effect of light emitting diodes (LEDs) on the in vitro induction and growth of bulblets of Lilium oriental hybrid 'Pesaro'. *Sci. Hortic.* **2002**, *94*, 365–370. [CrossRef]

37. Duong, T.N.; Hong, L.T.A.; Watanabe, H.; Goi, M.; Tanaka, M. Efficiency of a novel culture system by using light-emitting diode (LED) on in vitro and subsequent growth of micro-propagated banana plantlets. *Acta Hortic.* **2003**, *616*, 121–127.
38. Wang, H.; Gu, M.; Cui, J.; Shi, K.; Zhou, Y.; Yu, J. Effect of light quality on $CO_2$ assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. *J. Photochem. Photobiol. B Biol.* **2009**, *96*, 30–37. [CrossRef] [PubMed]
39. Lin, K.H.; Huang, M.Y.; Huang, W.D.; Hsu, M.H.; Yang, Z.W.; Yang, C.M. The effect of red, blue and white light emitting diodes on the growth, development and edible quality of hydroponically grown lettuce (*Lactusasativa* L. var. capitata). *Sci. Hortic.* **2013**, *150*, 86–91. [CrossRef]
40. Ma, G.; Zang, L.; Kato, M.; Yamawaki, K.; Kiriiwa, Y.; Yahata, M.; Ikoma, Y.; Matsumoto, H. Effect of blue light and red LED light irradiation on β-cryptoxanthin accumulation in the flavedo of citrus fruits. *J. Agric. Food Chem.* **2012**, *60*, 197–201. [CrossRef]
41. Hernandez, R.; Kubota, C. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. *Environ. Exp. Bot.* **2016**, *121*, 66–74. [CrossRef]
42. Johkan, M.; Shoji, K.; Goto, F.; Hahida, S.; Yoshihara, T. Blue light emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *HortScience* **2010**, *45*, 1809–1814. [CrossRef]
43. Glowacka, B.; Glowacka, B. Effect of light colour on the growth of tomato (*Lycopersicon esculentum* Mill.) transplant. *Acta Sci. Polonorum. Hortorum Cutlus* **2002**, *1*, 93–103.