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Original Article

Effect of Light Spectrum and Intensity on Growth of Grape (*Vitis vinifera*) Under *In Vitro* Conditions

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Abstract

Grape (*Vitis vinifera*) is the most important garden crop all over the world. Multiplication and breeding of most important garden and crop plants is based on the cell and tissue culture. Beside the medium composition, the incubation conditions also require to be optimized, too. Important factors in physical environmental of culture are including light, temperature and gas exchanging. The light plays a key role in the range of plant growth activation and is used as a source of energy in the photosynthesis process. Then it must be optimized for the most plant performance. In this study auxiliary buds of grape *cv*. Crimson Seedless have been grown in treatment of red (622-780 nm), blue (455-492 nm) and visible light (400-700 nm) with two intensity of 5000 and 2500 lux. The fastest growth of axillary bud is referred to the range of the red and visible light with 2500 lux (46.77 hour) and the most axillary bud growth was observed in the range of red light (65.77 mm). In the blue light the developed axis was the strongest. The rate of axillary bud photosynthesis in intensity 5000 lux (38.33 mm) achieved to the level of light saturation, and then dynamic light inhibitions (photoinhibition) and chronic were observed in this treatment.

Keywords: Light Spectrum, Grape, Photoinhibition, Non-photochemical Quenching Method

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Introduction

Grapevine (*Vitis* spp.) is one of the most important cultivated fruit crops world wide in all traditional methods, heterozygosity impediments, space, time and seed dormancy, limit performance [1]. Tissue culture is applied as a tool to produce grape primary material, such as disease-free, endemic and selective clones and new hybrid [2]. The axillary bud culture is the most application technique in micropropagation [3]. In some species shoot multiplication may occur spontaneously in a medium as growth regulator-free. However in most species it is necessary to add hormones for shoot induction. In addition to optimization of explant type and compounds of culture medium, optimization of incubation conditions such as light, temperature and gas exchange, also is necessary [4, 5]. The spectrum, photoperiod and intensity are three important light characters in plant tissue culture that can effect on plant activities and used as a source of energy in photosynthesis. In addition, light characterizations effects on cell differentiation and plant morphogenesis. Light or photoperiod may control dormancy, germination and some other physiological phenomena [6]. So it is necessary to pay attention to light components.

Since the middle of the 20th century, high-quality rootstocks have been selected for grape production because of their compact on growth habit, improved fruit pigmentation, earlier harvesting time and proven resistance to phyloxera [7]. Grape plantlets have commonly been cultured under low light intensity and high relative humidity and with sucrose or growth regulators supplemented in the culture medium. Thus, poor *in vitro* environments may limit their photosynthesis and growth [8]. When hairy roots of red beet (*Beta vulgaris* L.) are cultured under bioreactors, blue or far-red light qualities are more effective than conventional fluorescent lamps in enhancing not only carbohydrate accumulation but also betaxanthin and betacyanin contents [9, 10].

Kadkade & Japson (1987) have reported that betalain synthesis can be improved if one utilizes either 1:1 blue far-red light (B/Fr) or a higher ratio. Other researchers have also suggested that plant growth and morphogenesis are affected not only by light quality but also by phytohormone content [4]. Light is the energy source for photosynthesis and plant development.

Traditionally, fluorescent, metal halide, or inflorescent lamps have been used for *in vitro* plant production. Time courses for net photosynthetic rates (NPR) per plantlet were estimated by measuring the differences in CO_2 concentrations between the inside and outside of the culture vessel, taking into account the number of air exchanges and vessel air volume, but it is difficult to measure gas exchanges and probably with measurement error [8]. So we can use direct methods to estimate the rate of photosynthesis; for example, measurements of shoot growth and pigment content.

In this study for investigation of light quality and quantity on shoot growth, the auxiliary buds of Crimson Seedless variety of grape have been grown in red range (622-780 nm), blue (455-492nm) and visible (400-700 nm) light in two intensities (2500 and 5000 lux).

Materials and Methods

Plant material and culture conditions

In this research appropriate explants include young stems of grape (Crimson seedless variety) were collected. At first the stems were surface sterilized by 1.2% sodium hypochlorite solution with Tween 20 and shaking for 20 minutes. Then explants were rinsed four times in sterilized distilled water under laminar flow air cabinet. Then the stems with about 2-3 cm length with an auxiliary bud were cut and cultured.

After culture, explants were incubated at 25°C under appropriate photoperiod (16 h light and 8 h dark). The measurement of produced plantlets length, ranking and root scoring were done every week. To determine light intensity on shoot growth rate, the auxiliary buds have been grown in red light (622-780 nm), blue (455-492 nm) and visible light (400-700 nm in two intensities 2500 and 5000 lux). The experiment was carried out based on Completely Randomized Design (CRD) in 9 replications. The explants were cultured on MS (Murashige & Skoog) medium supplemented by 1mg/L Benzyl Amino Purine (BAP) and 7 g/L agar (pH 5.8).

Statistical Analysis

Statistical analysis was performed using the SAS system (Version 9). Means comparison was done by LSD test at 1% probability level.

Results and Discussion

The analysis of variance (Table1) showed there was significant differences between the light spectrums for growth initiation. The results indicated that this trait is depending on spectrum and light intensity. In other words, the growth initiation in different optical spectra indicated a significant differences between the spectra of red, blue and visible light (in 5000 and 2500 lux intensities).

Table 1. Optical spectrum effects variance analysis on auxiliary buds growth initiation in the Crimson seedless grape variety.

S.O.V	DF	MS
Optical spectrum	3	2003.56*
Error	32	125.31

*Significant ($p< 0.01$)

Mean comparison of growth initiation (Table 2) indicated that beginning the growth of the lateral buds, in red (46.77 h) light and visible light with 2500 lux (47.44 h)were faster than visible light with 5000 lux (72.55 h) and blue light (73.33 h).

In this case, the recording was done every week for four times. There was not significant differences between visible light with 5000 lux and blue light for growth initiation of auxiliary buds.

Optical spectrum effects variance analysis on auxiliary buds growth in different times in the Crimson seedless grape variety has been shown in Table 3. The statistical analysis showed that there was significant differences ($p<0.01$) among light spectrums for shoot growth in all weeks.

Table 2. Mean comparison of different lights on growth initiation of auxiliary buds in the Crimson seedless grape variety.

Optical spectrum	Mean (hours)
Red light (622-780 nm)	46.77 A
Visible light (2500 lux)	47.44 A
Visible light (5000 lux)	72.55 B
Blue light (455-492nm)	73.33 B

Table 3. Optical spectrum effects variance analysis on auxiliary buds growth in different times in the Crimson seedless grape variety.

S.O.V	D.F	MS			
		1st week	2nd week	3rd week	4th week
Optical spectrum	3	41.70**	1113.80**	1422.25**	1368.62**
Error	32	5.93	89.20	92.58	173.72

**Significant ($p< 0.01$)

The means comparison of optical spectrum on shoot growth during the different times in the Crimson grape variety has been demonstrated in Tables 4 to 7. The all means comparisons showed significant differences among optical spectrums for shoot growth. The shoots grown under spectrum of red light showed the highest growth in all stage of measurement (average 65.77 mm after four week). In order to evaluation of visible light spectrum in two intensities 2500 and 5000 lux, it was irradiated to the shoot. In overall, the statistical analysis showed that 2500 Lux was more efficient for shoot growth rate (Tables 4-7 and Fig. 2). Analysis details are as follow, at the begging of growth (first seven-day), there was no significant difference between two intensities of visible light for growth rate (Table 4). At the second seven-day, the visible spectrum with 5000 lux intensity (31.22 mm) was higher than 2500 lux (30.33 mm) for growth rate. Almost the difference was not statistically significant (Table 5). At the third seven-day, the shoots had the better growth in visible light 2500 lux (49.33 mm) rather than 5000 (35.88 mm) (Table 6). In the fourth week the growth rate of visible light with the intensity of 2500 (59.22 mm) has the significant higher than 5000 lux (38.33 mm). The growth of the shoots under blue light spectrum (455-492 nm) indicated that the explant growth in first, second and third weeks was at the minimum amount than other spectrums. However in the fourth week blue light showed better growth rate (46.66 mm) than the visible light with 5000 lux (38.33 mm) (Table 7). Cheng (2016) [11] showed that plant growth is inhibited in high intensities that this result is according to our funding.

When plants are exposed to high-intensity light, photosynthetic apparatus activation has been stopped [12]. Absorbing too much light, can cause light inhibitions (Photoinhibition) [8, 13, 14]. Plants usually appear light inhibitions [13, 14]. In general, plants absorb high light to power the photochemical reactions of photosynthesis. Nevertheless, this process carries with it the potential to harm the photosynthetic machinery, primarily photosystem II (PSII), thus causing photoinhibition.

Table 4. Mean comparison of optical spectrumon shoot growth during the first seven-day of the Crimson seedless grape varieties.

Optical spectrum	Mean (mm)
Red light	7.11 A
Visible light 2500 lux	5.33 BA
Visible light 5000 lux	3.66 BC
Blue light	2.11C

Table 5. Comparison mean spectral and light intensity on shoot growth during the second week of the Crimson seedless grape varieties.

Optical spectrum	Mean (mm)
Red light	46.11 A
Visible light 5000 lux	31.22 B
Visible light 2500 lux	30.33 CB
Blue light	19.00 C

Table 6. Comparison mean spectral and light intensity on shoot growth during the third week of the Crimson seedless grape varieties.

Optical spectrum	Mean (mm)
Red light	55.44 A
Visible light 2500 lux	49.33 A
Visible light 5000 lux	35.88 B
Blue light	27.77 B

Table 7. Comparison mean spectral and light intensity on shoot growth during the fourth week of the Crimson seedless grape varieties.

Optical spectrum	Mean (mm)
Red light	65.77 A
Visible light 2500 lux	59.22 BA
Visible light 5000 lux	38.33 C
Blue light	46.66 BC

This can, in turn, reduce photosynthetic activity, growth and productivity. Consequently plants have developed mechanisms that can rapidly and effectively repair photodamaged PSII; as a result, net photoinhibition only happens when the rate of damage exceeds that of the repair [11, 14]. To avoid net photoinhibition, plants have developed varied photoprotection mechanisms such as light avoidance related with the movement of leaves and chloroplasts; screening of photoradiation; reactive oxygen species (ROS) scavenging systems; dissipation of absorbed light energy as thermal energy (qE); cyclic electron flow (CEF) around photosystem I (PSI); and the photorespiratory pathway [11, 15, 14].

Photoinhibition may be occurred by other stresses too. It may happen in vary degrees. The plants that exposed to the temperature threshold (low and high), drought, nutrient stress and UV-B light, inhabitation may be accrued even in low light intensities [12, 14].

Light inhabitation may resulted by high or low temperature. For example, photosynthesis photoinhibitionin leaves of California wild grapes in high PPFD (Photosynthetic Photon Flux Density), in high and low temperature was more than average temperature. However, in the field with high PPFD light inhabitation was accrued despite the absent of other stress factors [12]. There are many cases about inhibition light. Optical inhibition has been reported in kiwi, willow leaves, cotton leaves, *hedera helix*, several species of tropical trees and cocoa leaves and California grape leaves [12, 14]. As has been shown mean comparison in Table 5, visible light with 5000 lux (31.22 mm) has the better growth than the visible light with the 2500 lux (30.33 mm). Nevertheless in mean comparison of third week (Table 6), growth rate of shoot in visible light with 2500 lux (49.33 mm) increased and even archived to the maximum rate of growth in red spectrum. This replacement may be due to saturation point after second week in visible light with 5000 lux intensity and light inhabitation occurred, photosynthesis reduced and finally growth decreased. When the leaves are exposed to more light than they need, second photosynthesis center (PSII) will be disabled and may be degraded often in the form ofinhibition inhabitation. In our experiment, the burn symptoms was absorbed instem and leaf tip in the visible spectrum 5000 and 2500 lux (Fig. 1).

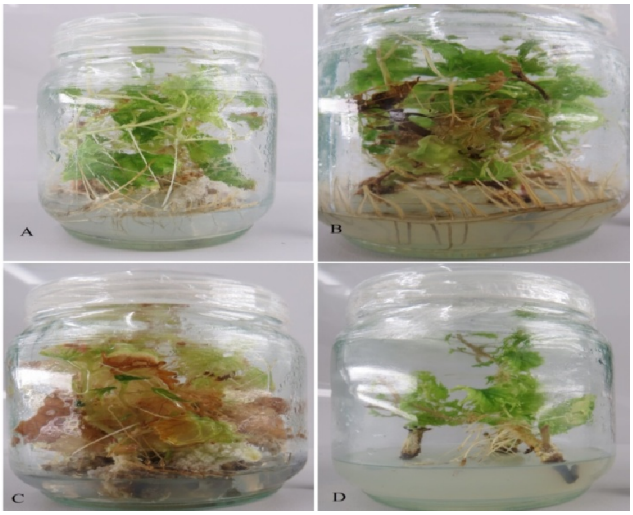

Figure 1. Appearance of *in vitro* plants in different optical spectrum. A) red spectrum B) visible light 2500 lux C) visible light 5000 lux D) blue spectrum.

Light inhibitory properties inleaves depend on rate of light that exposed to the plant. There are two kinds of light inhabitation, including: dynamic light inhibitory and chronic light inhibitory [14, 16].

Under extra light condition, dynamic light inhibition observed. In this situation the quantum efficiency decreases. However the maximum photosynthetic rate remains unchanged.

The dynamic light inhibitory is generated by deviation of the absorbed light energy to the distribution of heat, then quantum efficiency reduced. This reduction is often temporary and can return to the original value when the flux of photons reach below the saturation point [17].

Chronic light inhibitory created as a result of severe amounts of extra light that damage photosynthesis system. In this type of inhibition quantum, the efficiency and the

maximum rate of photosynthesis decreases. Conversely, in dynamic inhibitory light, these effects are relatively permanent and continue for weeks or months [16, 17]. In this research, due to chronic light inhabitation in visible light 5000 lux intensity, grapes is affected by chronic light inhibitory (Table 6 and 7) and in is shown that its growth rate is less than blue light (Fig. 2).

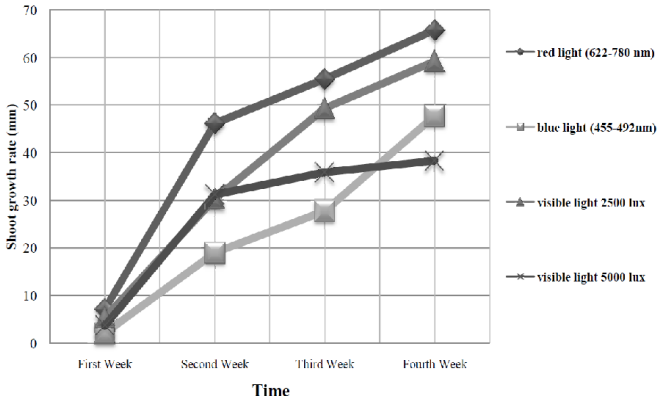

Figure 2. The effects of different optical spectrum on shoot growth rate in Crimson seedless grape variety.

Short term reductions of quantum efficiency are reflecting the protective mechanisms, while chronic inhibitory light indicates the actual destruction of chloroplasts as a result of more light or disability protection mechanisms. If chronic light inhibitory continues, causes loss of the plant [13].

In this study, when explants of grapes grown in the visible light of 5000 lux, after eight weeks burnt and died, that is consistent with result of Taiz and Zieger (2008) research.

As light inhabitation happened for explants grown in the visible light with 5000 lux, for visible light with 2500 intensity happened too. However its effects revealed slower in fourth week of growth. It should also be noted that in that more light conditions; leaves must dissipate excess light energy to prevent photosynthesis systems damaging. The plants may use several mechanisms to eliminate extra light such as non-photochemical landing (Non-photochemical quenching). The most important example of this type is removing absorbed light energy from the electrons chain to heat [13, 15]. Recovery from light inhibition depends on medium temperature [12, 14]. According to the closed culture dishes and a small thermal exchange with the surrounding environment, increasing temperature from appropriate level of growth even a few level, can have negative effect on fast growth of plant [14]. Explant from temperate regions grapes showed the best growth 20 to 28°C [15, 18]. Therefore extra lighting reduces photosynthesis directly,in addition, produced heat by Non-photochemical.

The resulted heat in closed environment of explants may be secondary factor to reduced shoot growth. This may be the reason of faster dynamic and then chronic optical inhibition of explants grown in the light spectrum with 5000 lux intensity than the other spectrum. This result is according to; Takahashi (2011), Huang (2016) andDüring (1998) reports [14,15, 19].

Conclusion

According to the obtained results in this study, light spectrum and intensity for optimal growth *in vitro* culture of grape is an important parameter. When the leaves are exposed to more light than they need, second photosynthesis center (PSII) will be disabled and may be degraded often in the form of inhabitation. Chronic light inhibitory is kind of light inhibitory. In this research, due to chronic light inhabitation in visible light 5000 lux intensity, grapes are affected by chronic light Inhibitory and the maximum rate of growth in red spectrum.

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References

1. Ganji, E., Temperate zone pomology education and promote agriculture. Tehran Press, 2011.
2. Kahrizi, D., Arminian, A., Masumi-Asl, A., Second edition. *In vitro* Plant Breeding, 2011, Razi University Press.
3. Chawla, H.S., Introduction to plant biotechnology, Science-Press, 2002.
4. Bonga, J.M., Aderkas, P.V., *In vitro* culture of trees, Ferdowsi University of Mashhad Press, 2004.
5. Chee, R., *In vitro* culture of *Vitis*: the effect of light spectrum, manganese sulfate and potassium iodide on morphogenesis. *Plant Cell Tissue Organ Cult*, 1986, Vol, 7, pp. 121-134.
6. Mancinelli, A.L., interaction between crptochrome and phytochrome in higher plant photomorphogenesis, *Amer J Bot*, 1989, Vol. 76, pp. 143-154.
7. Pierik, R. L. M., *In vitro* culture of higher plants,Ferdowsi University of Mashhad Press, 2004.
8. Kadkade, P. G., Jepson, H. Influence of light quality on organogenesis from the embryo derived callus of douglas fir (Pseudotsugamenziesii). *Plant Sci*, 1987, Vol. 13, pp. 67-73
9. Green, J. F., Muir R.M., An analysis of the role of potassium in the growth effects of cytokines, light and abscisic acid on cotyledon expansion. *Physio Plant*, 1979, Vol. 46, pp. 19-24.
10. Hart, J.W., Light and Plant Growth, Unwin Hyman London-Press. 1988.
11. Cheng, D.D., Zhang, Z.S., Sun, X.B., Zhao, M., Yusun, G., Chow, W.S. Photoinhibition and photoinhibition-like damage to the photosynthetic apparatus in tobacco leaves induced by *pseudomonas syringae pv*. Tabaco under light and dark conditions. *Plant Biol*, 2016, Vol. 10, pp. 723-726.
12. Pallardy, S.G., Physiology of woody plants, Aigah Press, 2008.
13. Taiz, L., Zieger, E., Plant Physiology Jahad University of Mashhad Press, 2008.
14. Takahashi, S., Badger, M.R., Photoprotection in plants: a new light on photosystem II damage. Review. *Trends in Plant Science*, 2011, Vol. 16(1), pp. 141-143.
15. Huang, W., Yang, Y. J., Hu, H., Zhang, S.B., Moderate photoinhibition of photosystem II protects photosystem I from Photodamage at chilling stress in *Tobacco Leaves*. *Front Plant Sci*, 2016, Vol. 7, pp. 182-184.

16. Smith, H., Sensing the light environment: The functions of the phytochrome family. *Photomrphogenesis in plants*, 1994, 2nd Ed.
17. Bortolin, R.C., Caregnato, F.F., Divan, A.M., Reginatto, F. H., Gelain, D.P., Moreira, J.C.F. Effects ochronicelevate dozone concentration on the redoxstate and fruit yield of red pepper plant *Capsicum baccatum*. *Ecotoxicol Environ Saf*. Vol. 100, 2014, pp. 114–121.
18. Chalupa, V., European hard woods. Cell and Tissue Culture in Forestry, Nijhoff Press, 1987, Vol. 3.
19. Düring, H., Photochemical and non-photochemical responses of glasshouse-grown grape to combined light and water stress, *Grape Res*. 1998, Vol. 37(1), pp. 1-4.