A Review of Artificial Light Technology; Leds on in Vitro Cultured Plant Morphology and Physiology

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ABSTRACT

Light strongly affects plant anatomical, physiological, morphological, and biochemical parameters of plant growth and development. Nowadays, artificial light technology is popular in agriculture system especially in vitro culture system. In this review, we aim to give an overview of the impacts of artificial light technology, which are LEDs on in vitro cultured plant morphology and physiology. The outcome shows the knowledge of using artificial light to promoted plant growth and development and how they affected.

Keywords: Light; Artificial light technology; Plant morphology; Plant physiology

1. INTRODUCTION

Light is one of the most important factors that affect the developing plant growth and development. Since seeds are germinate, they need to be response to their light environment. The responses of plants to light sensing from its quantity (fluence rate), quality (wavelength, i.e., color), direction, and duration (photoperiod) [1], [2]. Artificial lights sources vary in intensity, duration and spatial distribution. The light emitting diodes (LEDs) technology popularly used as supplementary light has shown great advancement in protected cultivation. One of the greatest challenges for the LEDs as alternative light source for greenhouses and closed environments is the diversity of the way experiments are conducted that often makes results difficult to compare. Morphology and physiology of grown plants are regulated by various micro-environmental factors such as light, temperature, humidity and carbon dioxide [3]. Light (spectral quality, photon flux density, and photoperiod) is an important factor among these and it generally influences the overall growth and development of in vitro plants [4]. Generally used light sources for culture of plants are fluorescent lamps; some research and commercial laboratories also use metal halide, sodium or incandescent lamps. The spectral range of these lamps vary from 350 to 750 nm (as shown in Figure 1), which contains mixture lights and affect the growth of cultured plants. Recently, light emitting diodes (LEDs) have been developed and used as an alternative light source for plant culture system because of their wavelength specificity and narrow bandwidth and minimum heating [5]. Red and blue lights have the greatest impact on plant growth because they are the major energy sources for photosynthetic CO_2 assimilation in plants.

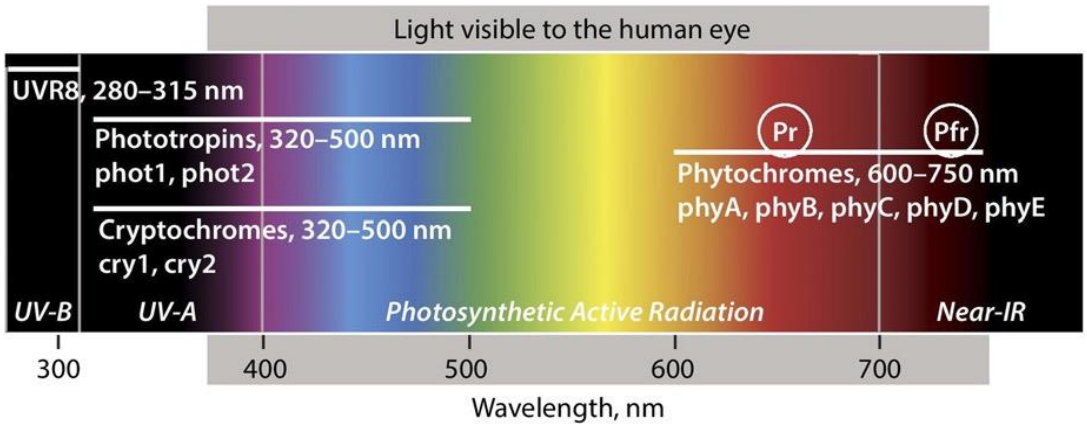

Figure 1. The range of wavelengths that are sensed by the main plant photoreceptors (phytochromes, cryptochromes, phototropins, and UVR8) allowing light-driven developmental adaptations (data from http://www.biologie.ens.fr/smdgs/spip.php?article57). [6]

2. EFFECT OF LIGHT QUALITY ON PLANT MORPHOLOGY AND PHYSIOLOGY

There are several reported about the light quality in micropropagation. [7] reviewed the use of blue and red LEDs has been commonly used for producers as these wavelengths are efficiently absorbed by the photosynthetic pigments as we known as chlorophylls, with red light being the most energy efficient in LED production. Both blue (420–450 nm) and red (600–700 nm) lights are absorbed by chlorophyll a (Chl a) which has its absorption peaks at 430 and 665 nm and chlorophyll b (Chl b) at 453 nm and 642 nm [8] (as shown in Figure 2. (A)). As the chlorophyll and nonchlorophyll pigments have different absorption spectra, the result is a composite absorption spectrum that is broadened such that a wider range of radiation is absorbed by plants [9] (Figure 2 (B)). The light scattering increases the probability of absorption drastically, which is demonstrated if a leaf is vacuum infiltrated by, e.g., water (as shown in Figure 2 (C)) [10]. The light absorption in leaves represents absorption in all pigments, including non-photosynthetic pigments. Since some of the absorbed energy will not be delivered to the reaction centers of the two photosystems, the relative quantum yield of photosynthesis (as shown in Figure 2 (D)) will deviate from the absorption spectrum of the leaf (as shown in Figure 2 (B)).

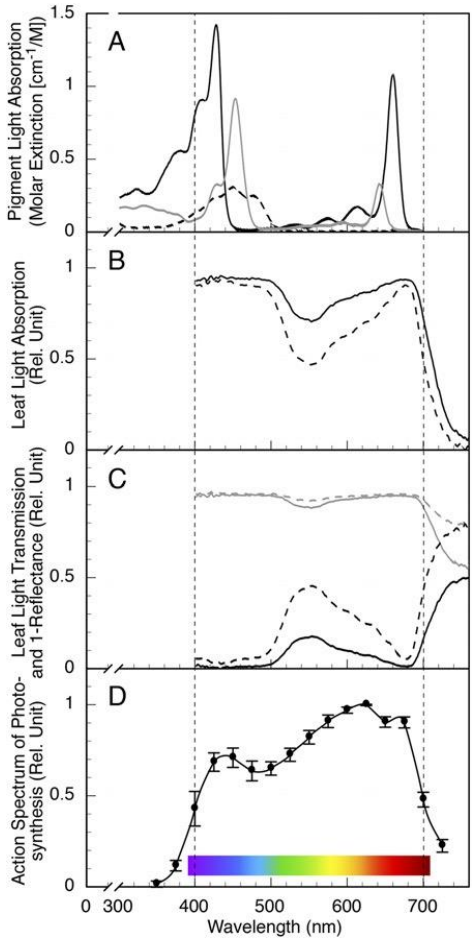

Figure 2. Spectrum for pigments and leaves. (A) Absorption spectrum of chlorophyll a (black line) and chlorophyll b (gray line) in diethyl ether, and beta-carotene (dashed line) in hexane based on data from http://omlc.ogi.edu/spectra/ PhotochemCAD/index.html. Other carotenoids like lutein and zeaxanthin have a similar absorption limit as beta-carotene [11] in the green range above 492 nm. (B) Light absorption in Chrysanthemum morifolium; fresh leaf (black line) and vacuum infiltrated by water (dashed line) to eliminate light scattering measured by a light integrating sphere (ASD Inc., Boulder, CO) and Avaspec- 2048 spectrometer (Avantes, Apeldoorn, The Netherlands). (C) 1-Reflectance (gray lines) and transmission (black lines) of the same fresh (solid lines) and vacuum infiltrated (dashed lines) leaves. (D) The relative quantum yield of photosynthesis of eight crop species (mean values ± SD) based on data from [12].

The influence of light quality on growth and development of in vitro grown Doritaenopsis hort was investigated. [13] Doritaenopsis hort plants were regenerate from leaf explants and supplied with four different light treatments; 1) fluorescent light (provided by white cool florescent lamps), 2) red LED (660 nm), 3) blue LED (450 nm), and red plus blue (1:1 photon flux density). The result showed that growth parameters were highest with plants grown under red plus blue light emitting diodes (LEDs). Leaf length was greater with the plants grown under red LED. Carbohydrate (starch, sucrose, glucose and fructose) and leaf pigment (chlorophylls and carotenoids) biosynthesis of the plants was significantly increased in plants grown under red plus blue LEDs compared to red or blue LED and fluorescent light treatments. This study suggested that the production of quality Doritaenopsis plants was possible by culturing the plants in vitro under a mixture of blue plus red light sources.

[14] They studied the effect of light quality on physiological transformation of in vitro Phalaenopsis 'Fortune Saltzman' seedlings. Phalaenopsis tissue culture seedlings were examined. They separated the seedling into three stages; stage I (seedlings of 1–2 cm in height with 1–2 leaves and 1–2 roots) tissue culture seedlings were grown under six different light qualities under a T5 fluorescent lamp: White, Red (610 nm), Red (658 nm), Blue (440 nm), Red (610 nm) + Blue (440 nm), and Red (658 nm) + Blue (440 nm). The result showed that after five months, cultured seedlings subjected to Blue (440 nm) treatment generated more leaves and presented higher levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content. On the other hand, seedlings subjected to the Red (658 nm) treatment had longer stems and leaves and higher fresh and dry weights than those subjected to other treatments. Root quantities increased under Red (658 nm), Blue (440 nm) + Red (610 nm), and Blue (440 nm) + Red (658 nm) treatments. This clearly shows that to enhance seedling growth through commercial production, Red (658 nm) should be applied.

[15] They studied on the effect of light quality on leaf production and development of in vitro cultured plants of Alternanthera brasiliana Kuntze. Light quality experiments were performed in growth chambers equipped with Sylvania Cool 60 F20T12 fluorescent tubes to provide different light qualities: red light, green light, white light, and blue light. White light and darkness conditions were used as control treatments. The result showed growth parameters including specific leaf mass, thickness, and leaf density were lowest in plants grown under red light. Blue light induced the largest number of leaves/plant, and the largest thickness and area of the leaf blade. Green and red lights induced the smallest leaf areas. The thickness of the abaxial-face epidermis and spongy parenchyma of the plants was significantly reduced in plants grown under red light. The thickness of the palisade parenchyma and upper epidermis were significantly increased in plants grown under blue light, compared to the other fluorescent-light treatments. In the dark and under red light, the mesophyll was homogenous; and in the dark and under green light, the leaves were more compact. Under blue light, the cells displayed the characteristic palisade morphology. The results showed that the increase of a specific parenchyma type was related to a specific spectral band. This study indicated that Alternanthera plants have strong morphological plasticity induced by light. The results suggested that high quality Alternanthera can be achieved by culturing the plants in vitro under a combination of blue and red light.

The effects of different light qualities on rapeseed (Brassica napus L.) plantlet growth and morphogenesis in vitro was study by [16]. The light sources generally used for in vitro plant cultures are fluorescent lamps. The plantlets were exposed to 60 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) for a 12 h photoperiod under the following six different light qualities: fluorescent lamps (FL), monochromic blue light-emitting diodes (LEDs) (B), monochromic red LED (R), and three mixtures of B plus R (3:1, 1:1, 1:3) LED. The proliferation rate was greater in plantlets that were cultured under B light than those under FL. The differentiation rate, fresh mass, dry mass, concentration of chlorophyll a, soluble sugar concentration, stem diameter, root activity, stomata frequency and transplantation survival rate were greater in plantlets that were cultured under B:R = 3:1 light than under FL. The concentration of starch and the spongy tissue length were higher in plantlets cultured under R light than those under FL. The B:R = 3:1 LED light was suitable for rapeseed plantlet growth in vitro and can be used as a priority light source in the rapeseed culture system according to its differentiation rate, proliferation rate, growth rate, and transplantation survival rate.

3. EFFECT OF LIGHT INTENSITY ON PLANT MORPHOLOGY AND PHYSIOLOGY

Light provides the energy needed for growth, photosynthesis and may influence plant productivity and seedling survival. Different light intensity is a key factor in the field, which varies seasonally, diurnally and spatially [17]. Phalaenopsis orchids have large well-developed leaves to utilize maximum light energy and when kept under high PPFD leaves show rapid photo bleaching.

[18] They were studied about the possible relationship between the effects of different levels of light intensity and the changes of antioxidant properties, Malondialdehyde (MDA) level, Lipoxygenase (LOX) activity, protein content and photosynthetic process during short term acclimatization in Phalaenopsis. Six months old in vitro grown plantlets were exposed to low light (LL-60 $\mu molm^{-2}\ s^{-1}$), intermediate light (IL-160 $\mu molm^{-2}\ s^{-1}$) and high light (HL-300 $\mu molm^{-2}\ s^{-1}$) photosynthetic photon flux density (PPFD), respectively under controlled condition. Plantlets exposed to HL intensity had lower level of Fv/Fm ratio than the LL grown plantlets during acclimatization. Regarding antioxidants enzymes, Superoxide dismutase (SOD) activity increased in leaves with increasing light intensity but light stress had no significant effect in roots. dehydro ascorbate reductase and monodehydro ascorbate content activities increased in LL and IL but decreased at HL. The Catalase (CAT) activity increased in both leaves and roots with increasing light intensity. While guaiacol peroxidase activity increased in roots, peroxidase activity was not detected in leaves. No significant change in glutathione reductase (GR) activity has been found at IL and HL, though it decreased significantly at LL compared to in vitro grown plantlets. There was an increase in ascorbate oxidase activity in leaves of about 50% at HL compared to in vitro grown plantlets, whereas no changes in roots were observed. glutathione S transferase activity showed pronounced stimulation in both leaves and roots of the plantlets exposed to HL compared to in vitro grown ones. Total leaf protein content increased in light stressed plantlets compared to in vitro grown plantlets. Leaf protein and LOX increased during light stress compared to in vitro grown plantlets suggesting that LOX mediated lipid peroxidation contributed to the oxidative damage occurring in the study. These results suggest that increase in enzyme activities were an adaptive response of the plantlets to higher amounts of reactive oxygen species (ROS) generated during acclimatization under light stress.

The effects of in vitro environmental conditions, ventilation of culture vessels and light level, on water loss control and photosynthetic capacity of Castanea sativa during in vitro culture were studied by [19]. C. sativa microshoots were cultured in ventilated (V) and non ventilated (NV) vessels, using two photon flux density (PFD) levels, 50 and 150 μmol photons $m^{-2}\ s^{-1}$ (L50 and L150, respectively). The use of ventilation and the increase in irradiance improved the performance of the microshoots with respect to traditional in vitro culture of C. sativa (NVL50). Microshoots grown under VL150 showed an increase in stomatal density and improved their functional characteristics, showing a more elliptical shape and lower percentage of stomata opening. This paralleled a significantly lower transpiration rate and stomatal conductance. Increasing light level and using ventilated vessels increased the microshoots capacity to harmlessly dissipate excess absorbed energy, water use and photosynthetic activity, resulting in a greater production of new microshoots. These improvements during in vitro culture generate microshoots with anatomical and functional characteristics similar to those observed in seedlings, which could help reduce the stress observed during ex vitro transfer.

[20] They studied on Photosynthetic response of in vitro guayule plants in low and highlights and the role of non-photochemical quenching in plant acclimation. Guayule (Parthenium argentatum L.) is a hypoallergenic latex-producing recalcitrant crop. During in vitro regeneration, the growth and the photosynthetic response of guayule was strongly affected by light intensities. Chlorophyll a (Chl-a) fluorescence was used to study the photosynthetic responses of in vitro grown guayule plants under low light (100 $\mu molm^{-2}\ s^{-1}$) and high light (1250 $\mu molm^{-2}\ s^{-1}$). In high light (HL), the shoot length was reduced and fresh and dry weights were enhanced, contrary to low light (LL) plant response. Total chlorophyll (Chl) and carotenoid contents based on fresh weight or leaf area were reduced by about 50% in HL compared to LL. Although maximum efficiency (Fv/Fm) of photosystem II (PSII) in the dark, electron transport rate (ETR-I), and quantum yield of photosystem I(PSI) were unaffected, the electron transport rate (ETR-II), quantum yield of PSII and non-photochemicalquenching (NPQ) were ~78–88% higher in HL than LL. There were no significant differences observed in malondialdehyde (MDA) content during regeneration of plants in either HL or LL. The higher NPQ in HL grown plants than LL grown plants suggests that NPQ plays an important role in photoprotection during acclimation of guayule plants when exposed to HL.

4. EFFECT OF PHOTOPERIOD IN MORPHOLOGY AND PHYSIOLOGY

Photoperiod is indicating the length of day or presence of light for plants grown under LED at total absence of natural light. Naturally, plants use photoreceptor proteins of phytochromes or cryptochromes to detect length of light, as well as absence of light or darkness [21], [22]. Changes of photoperiod affect to plant physiology such as, seed germination, plant growth and yield, while flowering of some temperate plant species depends on critical length of night [23]. [24] They study the effects of LED photoperiods and light qualities on the growth and chlorophyll fluorescence of Cunninghamia lanceolata (C. lanceolata) in vitro culture plantlets. In this study, plantlets were exposed to 20 $\mu molm^{-2}\ s^{-1}$ irradiance for three photoperiods, 8, 16, and 24 h under the three composite lights, 88.9% red+ 11.1% blue (R/B), 80.0% red+ 10.0% blue+ 10.0% purple (R/B/P), 72.7% red+ 9.1% blue+ 9.1% purple+ 9.1% green (R/B/P/G), as well as white light (12.7% red+ 3.9% blue+ 83.4% green, W) as control. The results showed that: plant height, dry weight, rooting rate, average root number, length, surface area and volume, chlorophyll, and chlorophyll fluorescence parameters were significantly affected by photoperiods, light qualities and their interactions. Plantlets subjected to photoperiod 16 h had longer root, higher height, rooting rate, root number, and the higher levels of chlorophyll, chlorophyll a/b, Y (II), qP, NPQ/4 and ETR_{II} compared to photoperiods 8 h and 24 h, while Fv/Fm during photoperiod 16 h was lower than 8 h and 24 h. Plantlets exposed to R/B/P/G generated more root and presented higher chlorophyll, Fv/Fo, Y (II), qP, and ETR_{II} than W during photoperiods 8 and 16 h. Total chlorophyll content and ETR_{II} were significant correlated with rooting rate, root length and root volume, while Fv/Fm and ETR_{II} were significant correlated with plant height, average root number and root surface area. 16-R/B/P/G is best for growing C. lanceolata plantlets in vitro. From the result, the experiment can conclude that the effectiveness of photoperiods and light qualities using LEDs for micropropagation of C. lanceolata. The best plantlets were harvested under 16-R/B/P/G treatment. And there was a correlation between the growth and the chlorophyll and chlorophyll fluorescence of their leaves under different photoperiod and light quality.

5. CONCLUSION

Light is an important factor that affect the developing plant growth and development especially plant morphology and physiology. Currently, artificial light plays an important role in agriculture technology and in vitro culture system. Artificial light technology also showed a great advantage to promote plant growth and development. Nowadays new trend of agriculture such as indoor crops, plant factory or vertical farm are also use artificial light supplied to plants, like that from LED, can be achieved through the light quality, light intensity and photoperiod to fulfil photosynthesis and other plant physiological functions which related to increase the quantity and quality of crop production. This review aims to show the effect of artificial light; LEDs to plant morphology and physiology to people who would like to use them in agriculture field.

REFERENCES

[1] Christie, J.M., Salomon, M., Nozue, K., Wada, M., & Briggs, W.R. (1999). "LOV (light, oxygen, or voltage) domains of the blue-light photoreceptor phototropin (nph1): Binding sites for the chromophore flavin mononucleotide." **Proc. Natl. Acad. Sci. USA.** 96, 8779–8783.

[2] Fankhauser, C., & Chory, J. (1997). "Light control of plant development". **Annu. Rev. Cell Dev. Biol.** 13, 203–229.

[3] Kozai, T., &. Smith, M.A.L. (1995). "Environmental control in the plant tissue culture: General introduction and overview." **In: Aitken-Christie J, Kozai T, Smith MAL (eds) Automation and environmental control in plant tissue culture**. Kluwer, Dordrecht, 301–418.

[4] Huges, K.W. (1981). "In vitro ecology: exogenous factors effecting growth and morphogenesis in plant culture system". **Environ Exp Bot.** 21, 281–288.

[5] Brown, C.S., Schuerger, A.C., & Sager, J.C. (1995). "Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting". **J Am Soc Hortic Sci**. 120, 808–813.

[6] Bowler, C. (2015). **Plant and Algal Genomic.** [online]. Available http://www.biologie.ens.fr/smdgs/spip.php?article57. Retrieved February 20, 2020.

[7] Ottosen, C.O., Ouzounis T., & Rosenqvist E. (2015). "Spectral Effects of Artificial Light on Plant Physiology and Secondary Metabolism: A Review". **Hortscience.** 50(8), 1128–1135.

[8] Sager, J.C., & McFarlane, J.C. (1997). "Plant growth chamber handbook, Radiation". In: R.W. Langhans and T.W. Tibbits (eds.). **Lowa Agriculture and Home Economics Experimental Station Special Report.** 1-29. Lowa State University Press, Ames, IA.

[9] Davies, K. (2004). "Plant pigments and their manipulation". **Ann. Plant Reviews 14**. Blackwell Publishing, Oxford, UK.

[10] DeLucia, E.H., Nelson, K., Vogelmann, T.C., & Smith, W.K. (1996). "Contribution of intercellular reflectance to photosynthesis in shade leaves". **Plant Cell Environ,** 19, 159–170.

[11] Koning, R.E. (1994). "Light. Plant Physiology Information" Website <http://plantphys.info/plant_ physiology/light.shtml>.

[12] McCree, K.J. (1972). "The action spectrum, absorptance and quantum yield of photosynthesis in crop plants". **Agr. Meteorol.** 9, 191–216.

[13] Shin, K.S., Murthy, H.N., Heo, J.W., Hahn, E.J., & Paek, K.Y. (2008). "The effect of light quality on the growth and development of in vitro cultured Doritaenopsis plants". **Acta Physiol Plant.** 30, 339–343.

[14] Anuchai, J., & Hsieh, C.H. (2017). "Effect of different light quality on physiological transformation of in vitro Phalaenopsis 'Fortune Saltzman' seedlings during growth period". **The Horticulture Journal.** 86(3), 395-402.

[15] Macedo, A.F., M. Vincius Leal-Costa, E. Schwartz Tavares, C. Luiz Salgueiro Lage, & M. Apparecida Esquibel. (2011). "The effect of light quality on leaf production and development of in vitro-cultured plants of Alternanthera brasiliana Kuntze". **Environmental and Experimental Botany.** 70, 43-50.

[16] Li, H., Tang, C., & Xu, Z. (2013). "The effects of different light qualities on rapeseed (Brassica napus L.) plantlet growth and morphogenesis in vitro". **Scientia Horticulturae.** 150, 117-124.

[17] Bjorkman, O., (1981). "Responses to different quantum densities". In: Lange, O.L., Nobel, P.S.,Osmond, C.B., Ziegler, H.(Eds.), **Encyclopedia of Plant Physiology. New series. Physiological Plant Ecology I, vol. 12A.** 57-108. Springer, Berlin, Heidelberg, New York.

[18] Ali, M.B., Hahn, E.J., & Paek, K.Y. (2005). "Effects of light intensities on antioxidant enzymes and malondialdehyde content during short-term acclimatization on micropropagated Phalaenopsis plantlet". **Environmental and Experimental Botany.** 54, 109–120.

[19] Saez, P.L., Bravob, L.A., Latsaguec, M.I., Sancheza, M.E., & Riosa, D.G. (2012). "Increased light intensity during in vitro culture improves water loss control and photosynthetic performance of Castanea sativa grown in ventilated vessels". **Scientia Horticulturae.** 138, 7–16.

[20] Turana, S., Kumara, S., & Cornishb, K. (2014). "Photosynthetic response of in vitro guayule plants in low and highlights and the role of non-photochemical quenching in plant acclimation". **Industrial Crops and Products.** 54, 266–271.

[21] Chia, P.L., & Kubota, C. (2010). End-of-day far-red light quality and dose requirements for tomato rootstock hypocotyl elongation. **HortScience**. 45: 1501-1506.

[22] Craig, D.S., & Runkle, E.S. (2016). "An intermediate phytochrome photoequilibria from night-interruption lighting optimally promotes flowering of several long-day plants". **Environmental and Experimental Botany.** 121: 132-138.

[23] Xu, Y., Ibrahim, M. I., & Harvey, P.J. (2016). "The influence of photoperiod and light intensity on the growth and photosynthesis of Dunaliella salina (chlorophyta) CCAP 19/30". **Plant Physiology and Biochemistry.** 106: 305-315.

[24] Xu, Y., Yang, M., Cheng, F., Liu S., & Liang, Y. (2020). "Effects of LED photoperiods and light qualities on in vitro growth and chlorophyll fluorescence of Cunninghamia lanceolata ". **BMC Plant Biology.** 20: 269.