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# SPECTRAL QUALITY AFFECTS MORPHOGENESIS ON ANTHURIUM PLANTLET DURING IN VITRO CULTURE

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**ABSTRACT**

This paper elucidates the effects of LEDs spectral on callus induction, proliferation and shoot development of anthurium plantlet derived from leaf explants. The research was conducted at the Ornamental Research Station, Fukuyama, Japan from January to August 2008. Three experimental series were designed to determine the effects of LED-based spectral compositions i.e. 100% red, 75% red + 25% blue, 50% red + 50% blue, 25% red + 75% blue and 100% blue LEDs on morphogenetic process of callus formation derived from leaf explants up to plantlet formation on two anthurium cultivars, Violeta and Pink Lady. The results showed no differences among cultivars tested but interaction of factors studied were found in all parameters observed. LEDs spectral gave significant influence on the morphogenetic processes from callus induction to complete plantlet formation. Progressive initial callus was promoted with the decrease of blue LEDs portion. Conversely, to proliferate globose to torpedo callus formation, more blue light was required than red LEDs. During shoot induction and formation, hastened shoot initiation and number of shoots were achieved in higher blue LEDs portions, but not in root formations.

Keywords: Anthurium, LEDs spectrals, morpho-genesis, plantlet, in vitro

## INTRODUCTION

Anthurium (*Anthurium andreanum* L.), grown as potted plant and as cut flower is one of the most important ornamentals in the world. More than 6,000 species that have been identified are mostly distributed under the tropical rain forests of Central America. These were used in the inter- and intra - specific hybridization producing unique and distinctive genotypes which are reflected in various leaf and flower colors and shapes in the existing commercial cultivars (Rosario, 1991). This complex genetic constitution led to some problems associated with the mass propagation of anthurium hybrids.

In vitro culture has been found to be an excellent tool for propagation in marketable quantities of plants with selected characters. This method guarantees the identical reproduction of the parents tested and selected, and prevents genotypic alteration which would occur after the generative multiplication (Chen *et al*., 2002). Successful in vitro propagation of anthurium mostly involved nutrient and hormone modification to accelerate cell development from intact explants (Budiarto and Handayati, 2007; da Silva *et al*., 2005; Martin *et al*., 2002; Rosario and Valenzuela, 1998). From this stage, morphogenesis and callus promotion for shoot development are being dealt with (Matsumoto *et al.,* 1998). Specific interactions between genotypes and chemicals added in the media have become a limiting factor in this phase.

Aside from genotype of the explants and optimum level of phytohormones within the media, plantlet morphogenesis in vitro is also primarily affected by the physical environment, such as spectral quality which affected not only the degree of endogenously induced hormone activities, but also the activated exogenous hormone within the media (Sanago *et al*., 1995). Variations on the optimum callus development, proliferation and formation of protocorm - like bodies were observed in *Cymbidium* cultured under different spectral regimes but the same types and concentrations of phytohormones in every stage of induction (Huan and Tanaka, 2004).

Among the morphogenesis-inducing spectrals, red and blue light were commonly used on in vitro-based modified technique using light emitting diode (LED). These were dispensable, since photosynthetic active radiations were felt in the range of these spectrals (Seabrook, 2005). With their small mass and volume, solid state construction, specific narrow-bandwidth wavelength emissions and longevity, LED-based spectral source might have specific control on the cell growth and development (Kurilcik *et al*., 2007).

The use of LED-based modified spectral on in vitro culture of anthurium has never been reported. In this paper, the effects of spectral quality on the callus induction and proliferation, shoot development and complete plantlet formation from leaf explants of two commercial anthurium cultivars were investigated. The specific requirements of spectral compositions on every induction stage are elucidated and described for establishing standard propagation procedure for the plant.

## MATERIALS AND METHODS

The research was conducted in the tissue culture laboratory of the Ornamental Research Station, Fukuyama, Japan from January to August 2008. Three experimental series were designed to determine the effects of LED-based spectral compositions namely,100% red, 75% red + 25% blue, 50% red + 50% blue, 25% red + 75% blue and 100% blue LEDs on the morphogenesis of callus and plantlet formation in leaf explants of two anthurium cultivars, Violeta and Pink Lady. The LED spectral treatments were arranged in an apparatus of white acrylic plastic boxes named LED Pack 4 equipped with LED boards mounted on the ceiling. Two types of LED boards were developed; the first one was comprised of 600 individual red LEDs (wavelength 660 nm from GaN-GL5UR3KI 3cd, Sharp Electric Ltd, Japan) and 600 individual blue LEDs (wavelength 450 nm from GaAIA-NLPB 1 cd from Nichia Chemicals Ltd, Japan). The second type was comprised of 1200 individual red LEDs. The ratios of red and blue LEDs as well as the intensity of light were adjusted using PA36-2A regulated DC power supply (Kenwood TMI Corp, Japan and LI-250 light meter (LI-COR Inc., USA).

Anthurium plants of both cultivars were collected from commercial nurseries. They were replanted into 15 cm pots. The plants were then placed into the growth chamber and h provided with 24 C day/18 C night temperature and 16 h photoperiod for 4 weeks. Twice a week foliar applications of nitrogen fertilizer were made to promote new leaf development. The new young leaves then served as the source of explants. The explants were collected, cut and disinfected in 0.1 % sodium hypochlorite with two drops of wetting agent (Tween 20/100 ml) for 2 min. The leaves were then sterilized with 80% ethanol for one min and rinsed twice with autoclaved sterile water. After quick drying with sterile papers, the leaves were cut into 1 x 1 cm size Each piece was inoculated into hormone-free media for 7 days to ensuring no contamination existed in the cultures.

The explants were transferred into the modified Nitsch and Nitsch media supplemented with 1 mg/l 2,4-D + 1 mg/l Kinetin + 1 mg/l BA and placed into the corresponding LEDs spectral boxes treatments. The intensity of LEDs in each box was maintained at 45 μ mol $m^{-2}$ $s^{-1}$ with a 16-h photoperiod under 16 - 18 C daily temperature. Callus development was observed after 30 days of incubation. The best callus developments on the first experiment were then transferred into the same media and placed under spectral treatment boxes. Observations of torpedo callus formation were made 50 days after incubation. Subsequently, the experiments on the effects of LED-based spectral treatments on the shoot initiation and formation until complete plantlet development were conducted The best callus derived from the second experiment (60 days after incubation) was used for further shoot proliferation and development. About 0.5 cm - callus was sliced and deflasked into fresh but same media. One sliced callus was inoculated per culture flask and placed into corresponding LED spectral box treatments. The cultures were maintained in modified environment similar to the first experiment. Observation on the fully developed plantlets was conducted after 60 days after which and they transferred outside for acclimatization. All the data gathered were analyzed using F test (α = 5%), and the differences among treatments were tested using Least Significant Different (LSD 5%).

## RESULTS AND DISCUSSION

LEDs spectral quality significantly affected callus induction-proliferation, shoot initiation and complete plantlet formation in anthurium in vitro culture. The two anthurium cultivars used in this study did not show any differences on all parameters evaluated (Table 1). However, except for the mortality of the explants, LEDs spectral quail significantly affected callus development and formation, initiation and number of shoots and roots.

### Effects of LEDs Spectral on Callus Initiation and Proliferation

Initial callus development at 30 days after incubation was more progressively detected on the explants under 100% red LEDs followed by those under PGF (Plant Growth Flourescent), 75% red + 25% blue and 50% red + 50% blue. Explants under 100% blue LEDs showed the least callus development (Table 2).

In the second experiment, callus of 'Violeta' derived from 100% red LED were transferred into the same but fresh media to observe further callus development under the PGF treatments. In contrast with initial callus development on the first experiment, the further callus development after 50 days incubation was merely observed under 100 % blue and 25 % red + 75 % blue LEDs. The least torpedo callus was observed on the initial callus stored at 100 % red LEDs (Figure 1 A.).

Table 1. Variance analysis of LEDs spectral quality on callus induction, proliferation, and complete plantlet formation in vitro culture of two anthurium cultivars.

| Source of variance | Callus induction and proliferation | | | Plantlet formation | | |
|---|---|---|---|---|---|---|
| | Percentage developed callus | Percentage torpedo callus formation | Mortality of explants | Shoot initiation | Number of shoots | Number of roots |
| Replication | ns | ns | ns | ns | ns | ns |
| LEDs spectral quality (A) | * | * | ns | * | * | * |
| Anthurium cultivars (B) | ns | ns | ns | ns | ns | ns |
| Interactions (A x B) | ns | ns | ns | ns | ns | ns |
| CV (%) | 11.61 | 12.37 | 11.71 | 10.26 | 14.23 | 13.31 |

Remarks: '*' = significant, and ns = not significant (LSD, 5 %)

Table 2. Callus development, percent torpedo callus formation and mortality of anthurium explants as affected by LEDs spectral quality

| Light spectral quality | Percent developed callus * | Percent of torpedo callus formation ** | Mortality of explants ** |
|---|---|---|---|
| 100 % red | 60.3a | 17.5c | 21.5 a |
| 75 % red + 25 % blue | 42.1b | 32.6b | 19.4 a |
| 50 % red + 50 % blue | 35.7bc | 31.8b | 22.7 a |
| 25 % red + 75 % blue | 27.6c | 45.6a | 21.5 a |
| 100 % blue | 22.9c | 46.3a | 20.6 a |

Remarks: - *, **) Values followed by different letters in the same column differ significantly by LSD 5 %
- *) Taken at 30 days after incubation
- **) Taken at after 50 days incubation

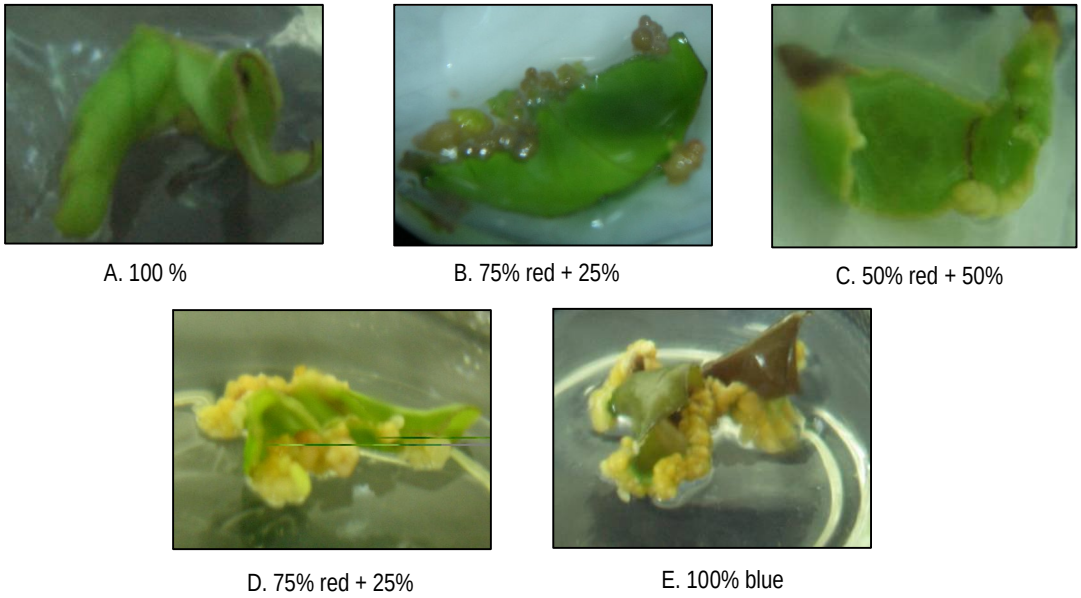



Figure 1A-E. Callus development in explants of *Anthurium* 'Violeta' subjected to different plant growth flouresence (PGF) for 50 days.

The different spectral regimes for optimum cell divisions and development of proliferated callus to torpedo buds inferred that cell physiological regulation was almost always interact with physical environment such as light. During in vitro culture, when an explant is transferred into nutritive media, the photoautotroph regulation is diverted (da Silva and Debergh, 1997). The existence of photosynthetic active radiation (PAR) that mainly falls on the wave length of 400 to 630 nm (blue) during this stage would still affect membrane configuration by eliciting electron transfer rate or heat release on chlorophyll (Schmid *et al*., 1987). At the stage when cell adaptation from nutrient transport system to direct absorption from media occurs, these conditions would affect the cell physiological system during photosystem II (PS II). The over come electron elicitation could not be facilitated in PS II as photon, since the efficiency of generated-energy of cell is in the lowest value (Drozdova *et al*., 2001). This prolonged stage contributed to the loss of functional continuity between photon harvest and energy processing in PS II (Jerzy *et. al.,* 2004). These would be the most putative mechanism in relation to the decrease of developed callus in anthurium leaf explants in consonance with the increase of blue light portion during callus induction. While the negligible differences on mortality of explants among the LEDs spectral treatments (Table 2) might refer to the intact explant being planted.

After certain stage callus development, spectral treatments gave different impacts on further callus development. Blue light induced further cell proliferation up to torpedo callus formation; the least was observed under red light (Figure 1 A-E). When the cells have become adapted to the modified environment including nutrient uptake, they retained their integrity on the physiological state (Jao and Fang, 2004a). The cell photomorphogenetic responses that were facilitated through interchangeably photoreceptors, cryptochrome and phytochrome might play important roles in light absorption and made use of photon flux to maintain cell physiological process (Patil *et al.,* 2001) by interfering with exogenous hormones within the media. In this experiment, the percentage of torpedo callus formation was highest in explants subjected to 100% blue light. Similar findings on the action of blue absorbing photoreceptor on other crops, like strawberry

(Biswas *et al.*, 2007; Miranda and Williams, 2007), petunia (Kuboda *et al.,* 2000) and potato (Jao and Fang, 2004b) have been reported. However, there is a lack of information on the adverse impact of red light at this stage.

**Shoot and full development of plantlets under different LED's spectral regimes**

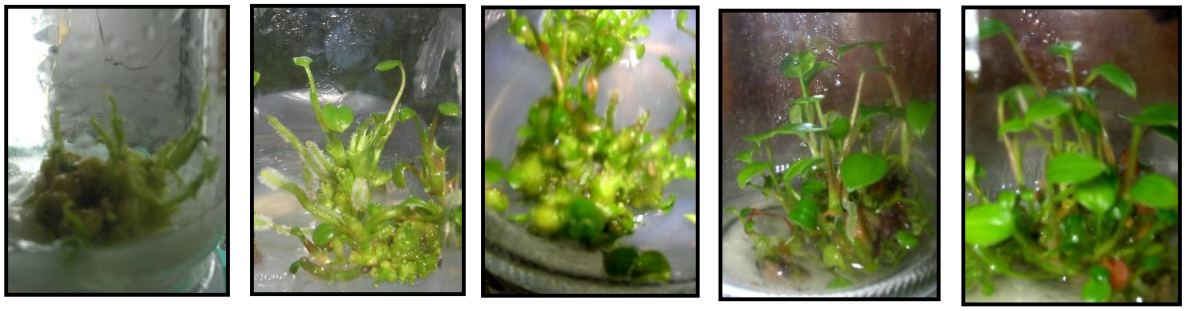
Shoot initiation, number of shoots and roots of anthurium plantlets subjected to different LEDs spectral regimes are presented in Table 3. Shoot initiation from torpedo callus was hastened by exposure to higher percentage of red than blue light but there were more shoots developed when exposed to higher percentage of blue than red light. Significant differences in the number of roots were observed in light treatment regimes. There were more roots in cultures exposed to higher percentage of red light with the least number developed under blue light. The anthurium plantlet formations under different light spectrals are presented in Figure 2.

The different optimum response of shoots and roots of plantlet to spectral regimes inferred that plantletshad different mechanism to control growth directions in respect to spectral available to them. In strawberry, the low intensity of PAR (remarkably blue light) induced root system (Passey *et al*., 2003) and either micro tuber formation in potato (Alix *et al*., 2001). The impact of blue illumination on shoot growth is due to several pertinent factors. The low PAR would subsequently decrease photosynthetic rate of plantlet, thus efficiency of process underwent to non shoot and leaves organs, roots (Naoya *et al.,* 2002) and prolonged treatment might cause shoot growth retardation.

Table 3. Effect of LEDs spectral quality on shoot and full development of anthurium plantlets 60 days after initial culture

| Light spectral quality | Shoot initiation (DAI) | Number of shoots | Number of roots |
|---|---|---|---|
| 100 % red | 46.7a | 12.4 c | 33.5a |
| 75 % red + 25 % blue | 44.1a | 15.7 bc | 31.4a |
| 50 % red + 50 % blue | 35.7b | 23.1 b | 24.7b |
| 25 % red + 75 % blue | 30.2bc | 33.7 a | 15.5cd |
| 100 % blue | 27.3c | 35.3 a | 12.4d |

Remarks : - Values followed by different letters in the same column differ significantly , LSD 5 %.
- DAI = days after incubation



100% red | 75% red + 25% blue | 50% red + 50% blue | 25% red + 75% blue | 100% blue

Figure 2. Plantlet development of 'Violeta' culture in vitro under different LEDs spectral light 60 days after incubation

Hormonal imbalance within the plants is another link to the mechanism. The hormone in the media could not interact solely in growth reorientation without conducive and defined physiological integrity within the plant. The low PAR was considered as a stressed environment, thus a signal for modified physiological system had made the plant proceed to organ development for self defense mechanism (Aksenova *et al.,* 2003). During these stages, hormonal interfering concentration decreased from shoot to root induction (Jackson, 1999), thus root developmental process were exhibited.

The results of this study together with the findings of other authors suggest that the response of cultured tissues to different light conditions might be dependent on species or even clonal specificities as well as specific light conditions. LEDs therefore, could be used to improve callus cultures and shoot-root inductions in anthurium during in vitro culture. It was reasonable to expect LED irradiation system could also be used in the micro-propagation of other ornamentals in the future.

## CONCLUSIONS

LEDs spectral quality affected plantlet morphogenesis of anthurium during in vitro culture. Initial callus induction in leaf explants was promoted with decrease of blue light portion. Blue LEDs were required more than red in the proliferation of globose to torpedo callus formations. During shoot induction and formation, hastened shoot initiation and increased number of shoots were achieved in higher blue LEDs portions. The root formation was progressively induced under higher portion of red LEDs.

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