Growth and Morphogenesis of *Protea cynaroides* L. Microshoots Cultured under Light-Emitting Diodes (LEDs)

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Abstract

Growth and morphogenesis of *Protea cynaroides* L. explants in vitro are relatively slow. Besides being essential for photosynthesis, light quality also plays an important role in plant morphogenesis. In this study, the effects of light quality on the growth of *P. cynaroides* apical shoots cultured under light-emitting diodes (LEDs) were investigated. Three light treatments were used: conventional cool white fluorescent lamps (FL) (control), far-red (FR) LEDs (770 nm), and red + far-red (R + FR) (1:1 spectral energy) LEDs (640 + 770 nm). Two types of growth media were used: half-strength Murashige and Skoog (MS) medium supplemented with either 0.1 mg L^{-1} naphthalene acetic acid (NAA) or 2 mg L^{-1} zeatin and 0.5 mg L^{-1} indole-3-acetic acid (IAA). After 35 days in culture, chlorophyll content (SPAD value) of apical shoots was lowest in explants cultured in growth medium containing 2 mg L^{-1} zeatin and 0.5 mg L^{-1} IAA, and irradiated by FR LEDs. Significantly higher chlorophyll content was detected in explants cultured under R + FR LEDs on medium containing 0.1 mg L^{-1} NAA, which was similar to those grown on medium containing 2 mg L^{-1} zeatin and 0.5 mg L^{-1} IAA under white fluorescent lamps. Under white light, the bud weight of apical buds cultured on media supplemented with 2 mg L^{-1} zeatin and 0.5 mg L^{-1} IAA was significantly higher than the other treatments. The highest number of buds was observed on shoots irradiated by white light and R + FR LEDs, cultured on media containing 2 mg L^{-1} zeatin and 0.5 mg L^{-1}, and 0.1 mg L^{-1} NAA, respectively. Explants irradiated by FR LEDs on medium containing 0.1 mg L^{-1} NAA produced the least number of buds. Results suggest that irradiation of *P. cynaroides* explants by LEDs containing red light induces the growth of new buds in the absence of zeatin.

INTRODUCTION

Protea cynaroides is one of the most important members of the *Proteaceae* family. *P. cynaroides* plants show great variation in nature with many different sizes, colours and flowering times. It is well known for its attractive flowerhead, and is commonly used as a cut flower. In recent years, production areas for proteas have been expanding in the Northern Hemisphere, including Portugal, Madeira Island, Israel, Canary Islands and France (Thillerot et al., 2006).

In vitro propagation techniques are widely used to propagate numerous economically important plants. Under in vitro conditions, growers are able to mass-produce plants in a relatively short period of time. In addition, in vitro propagation is also used to overcome problems that are found in traditional propagation, such as poor root formation of cuttings, slow growth rates, and susceptibility to diseases.

Although the in vitro establishment of *P. cynaroides* nodal shoot segments (Ben-Jaacov and Jacobs, 1981; Wu and du Toit, 2004) and apical buds (Thillerot et al., 2006) have been successful, no information is available on how to further stimulate the growth of *P. cynaroides* explants in vitro. Light is an important stimulus for plant growth and morphogenesis. Cool white fluorescent lamps are commonly used as a light source for explants in vitro. Light-emitting diodes (LEDs) on the other hand are attracting interest as an alternative light source for in vitro propagation. Wavelength specificity and the ability

to adjust its light intensity are the main advantages of LEDs (Okamoto et al., 1997). Other benefits of LEDs are their low thermal output and long life span. Red LEDs (620-680 nm) at various intensities are most commonly used to study their effects on plant growth and morphogenesis. Far-red LEDs (735 nm) have been reported to stimulate stem elongation in explants (Kim et al., 2004). This study was conducted to investigate the effects of different light sources and growth regulators on the vegetative growth of *P. cynaroides* explants.

MATERIALS AND METHODS

Plant Material

Embryos were excised from the seed coat and germinated in Murashige and Skoog (MS) (Murashige and Skoog, 1962) basal medium. Germination of the embryos was done according to Wu et al. (2007). Over a 12-month period, the seedlings were repeatedly subcultured to fresh media to increase shoot growth. The medium contained half-strength MS medium supplemented with 100 mg L^{-1} myo-inositol, 100 mg L^{-1} ascorbic acid (filter-sterilized), 0.5 mg L^{-1} benzyladenine (BA), 0.1 mg L^{-1} naphthalene acetic acid (NAA), and 2 mg L^{-1} silver nitrate. The medium also included activated charcoal (2 mg L^{-1}), sucrose (30 g L^{-1}), and agar (9 g L^{-1}). Apical shoots (10 mm) consisting of a single bud and four leaves were removed from micro-shoots and used as explants in this study.

Growth Medium and Light Quality Treatments

Two types of growth media were used: Half-strength MS media containing either 0.1 mg L^{-1} 1-naphthalene acetic acid (NAA) or 2 mg L^{-1} zeatin and 0.5 mg L^{-1} indole-3-acetic acid (IAA). All media also contained 30 g L^{-1} sucrose and 9 g L^{-1} agar. The pH of the media was adjusted to 5.5 before autoclaving for 25 min. The explants were placed on growth media in glass test tubes (one per tube), and cultured in LED lighting systems. The lighting systems were constructed from aluminium boxes (50 cm (L) × 50 cm (W) × 45 cm (H)), containing different types of LEDs. The explants were irradiated with conventional cool white fluorescent lamps (FL) (control), far-red (FR) (770 nm) LEDs, or red + far-red (R + FR) (640 + 770 nm) (1:1 spectral energy) LEDs. The LEDs were purchased from East Group Lighting Technology Co. Ltd. (Chinese Taipei). Three hundred red or far-red LEDs spaced 2 cm apart, were placed on the cover of each box. A temperature sensor, timer and two fans were also installed on each lighting system. The LED lighting systems were placed in a growth room throughout the entire duration of the experiment. The photon flux densities (PFD) for the light treatments were adjusted to 36 $\mu mol\ m^{-2}\ s^{-1}$. The photoperiod and temperature of the growth room was maintained at 16 h and 25±2°C, respectively. The wavelengths emitted by the LEDs and fluorescent lamps were measured with a spectro-radiometer (ILT900, International Light Technologies Inc., USA) (Fig. 1). The chlorophyll content (SPAD value) of the original four leaves of each explant was measured with a chlorophyll meter (SPAD-502, Minolta Co., Ltd., Japan).

Statistical Analysis

One explant per test tube, with ten replications per treatment was used. A completely randomized designed was used in all treatments. Data for chlorophyll concentration, mean apical bud fresh weight, mean number of new bud formation, and mean number of new leaf formation, were collected after 35 days in culture. Treatment means were separated using Duncan's Multiple Range test. Differences were considered significant when $P < 0.05$. Statistical analyses were done using the Statistical Analysis System (SAS) program.

RESULTS AND DISCUSSION

Results showed that similar levels of chlorophyll content were found in leaves of explants cultured under different light sources (Fig. 2A). However, significant differences were detected between chlorophyll levels in explants irradiated by conventional

fluorescent lamps and far-red LEDs grown in the same medium (2 mg L^{-1} zeatin, 0.5 mg L^{-1} IAA). Similarly, differences were found between explants cultured under R + FR LEDs (0.1 NAA mg L^{-1}) and far-red LEDs (2 mg L^{-1} zeatin, 0.5 mg L^{-1} IAA). On the other hand, chlorophyll content in explants irradiated by R + FR LEDs was found to be similar to those exposed to white fluorescent light. This is in agreement with a study by Kim et al. (2004) where no differences in chlorophyll content (SPAD value) were found between chrysanthemum explants cultured under white fluorescent light and R + FR LEDs.

Bud growth of explants, as indicated by apical bud weight (Fig. 2B), grown in media containing 2 mg L^{-1} zeatin and 0.5 mg L^{-1} IAA, and cultured under white fluorescent light were significantly higher than all the other treatments. Stem elongation (Kim et al., 2004) and peduncle elongation (Fukuda et al., 2002) have been reported in plants exposed to far-red LEDs. Similarly, R + FR LEDs were shown to stimulate shoot elongation in *Azorina vidalii* (Moreira da Silva and Debergh, 1997). However, inhibition of stem growth was reported in marigold and salivia cultured under red LEDs (Heo et al., 2002). In our study, although bud growth was observed in explants under R + FR LEDs and FR LEDs, the shoots and buds were not particularly elongated (data not shown). Findings from these studies suggest that the effects of red and far-red LEDs on stem elongation are not consistent. Their effects seem to vary according to R:FR ratio, intensity, and plant species.

The highest number of adventitious or axillary buds formed on apical shoots was observed on explants cultured under white fluorescent lamp (2 mg L^{-1} zeatin and 0.5 mg L^{-1} IAA) and R + FR LEDs (0.5 mg L^{-1} IAA) (Fig. 2C), while the lowest number of new buds was formed on apical shoots grown on medium containing 0.1 mg L^{-1} NAA under FR LEDs. Compared to FR LEDs, the inclusion of red LEDs (R + FR LEDs) on the same medium (0.1 mg L^{-1} NAA) significantly increased new bud formation. Furthermore, the number of new bud formation under R + FR LEDs on medium without zeatin (0.1 mg L^{-1} NAA) was similar to those irradiated by FL on medium containing zeatin (2 mg L^{-1} zeatin and 0.5 mg L^{-1} IAA). This suggests that irradiation of *P. cynroides* explants by red LEDs induces the growth of new buds in the absence of zeatin.

All apical shoots produced new leaves throughout the duration of this study. The number of new leaves formed on the explants was similar in all light and media treatments (Fig. 2D). Similar results were reported by Kurilčik et al. (2008), where the use of R + FR LEDs did not significantly affect the growth of chrysanthemum leaves, and were similar to conventional white lamps.

Figure 3 shows the growth of the apical shoots in all treatments. Overall, significant growth of apical buds was observed in growth medium containing 2 mg L^{-1} zeatin and 0.5 mg L^{-1} IAA under white fluorescent light (Fig. 3B). Leaf browning was observed in explants grown under far-red LEDs (Fig. 3C, D). The formation of new buds on explants irradiated by white light (Fig. 3G), R + FR LEDs (Fig. 3H), and FR LEDs (Fig. 3I) were clearly visible after 35 days in culture. No rooting was observed in any of the explants.

The results of this study showed that the growth of *P. cynaroides* apical shoots was influenced by light quality and growth regulators. Explants irradiated by far-red LEDs alone contained the lowest chlorophyll content, and produced the lowest number of buds and leaves. In terms of new bud formation, results indicated that explants exposed to R + FR light performed similarly to those cultured under white light. These results suggest that R + FR LEDs have the potential to be used as a light source for *P. cynaroides* microshoots, however, further studies are needed.

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Figures

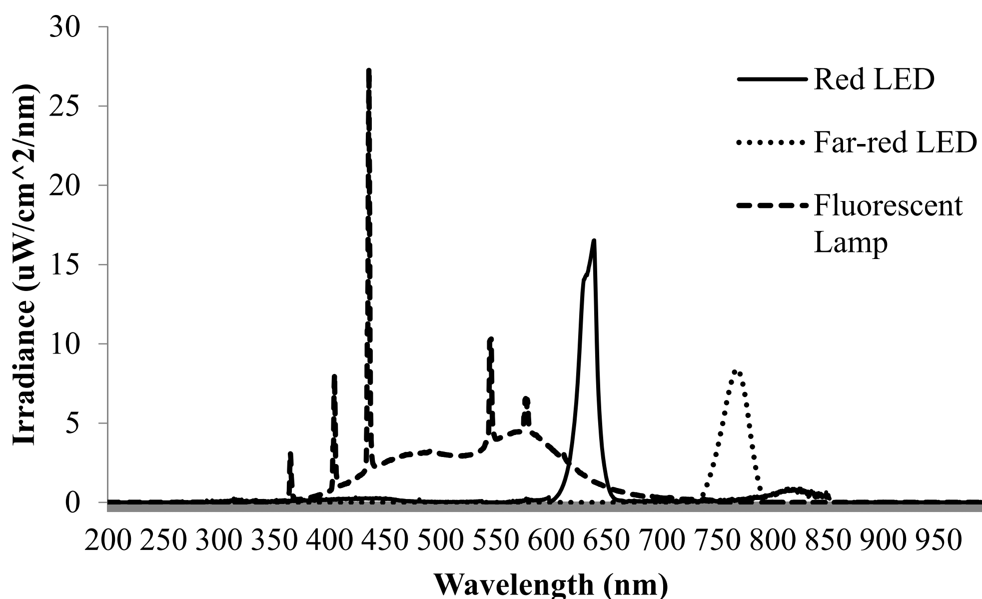

Fig. 1. Spectral distribution of LEDs and a fluorescent lamp.

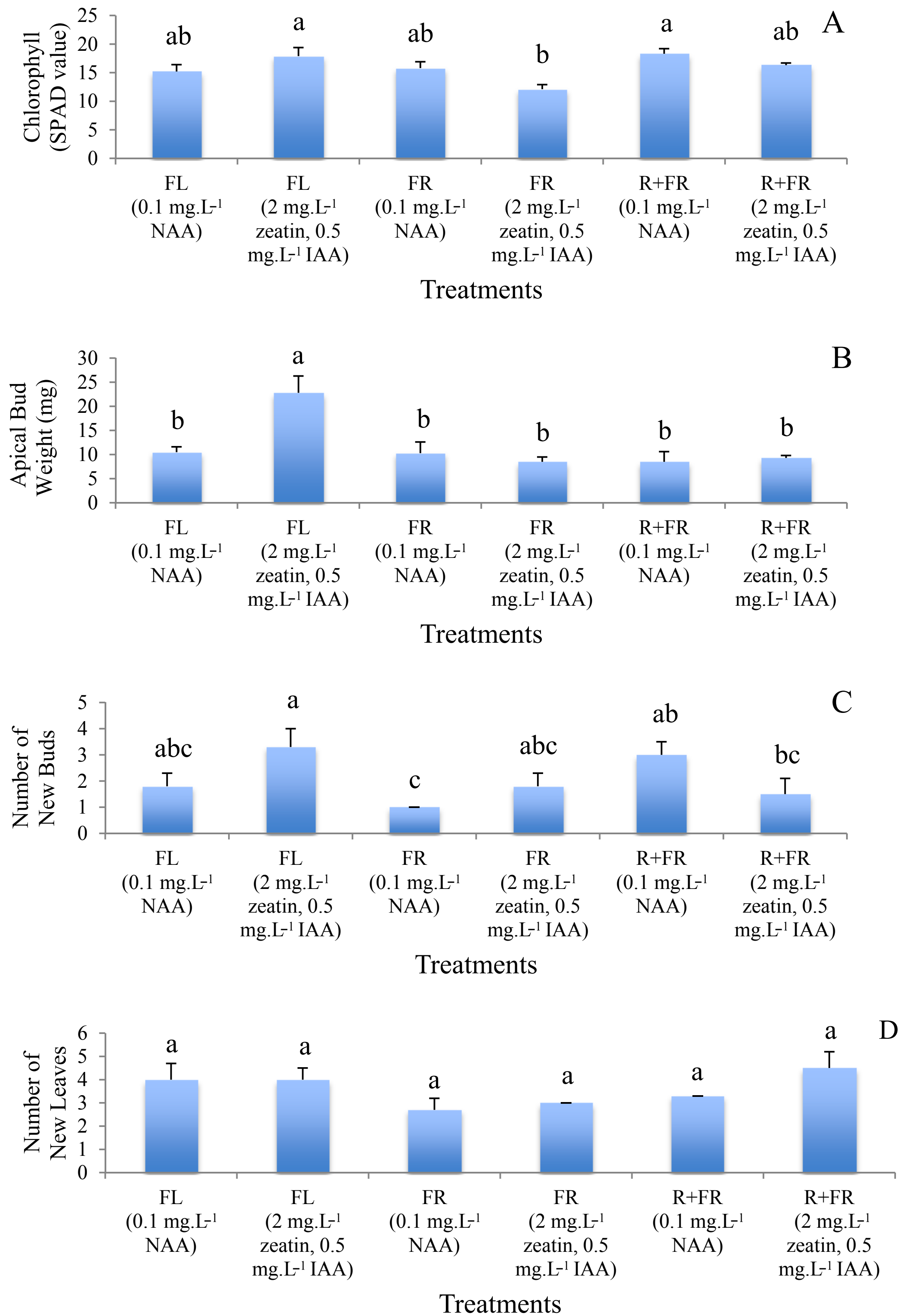

Fig. 2. Growth of microshoots under different light quality and media treatments, after 35 days in culture. (A) Chlorophyll content (measured with Minolta SPAD-502; average values of four leaves); (B) Apical bud weight; (C) Number of new buds formed; (D) Number of new leaves formed. FL = fluorescent lamp; FR = far-red light; R = red light. Treatment means with different letters are significantly different based on Duncan's Multiple Range test ($P < 0.05$).

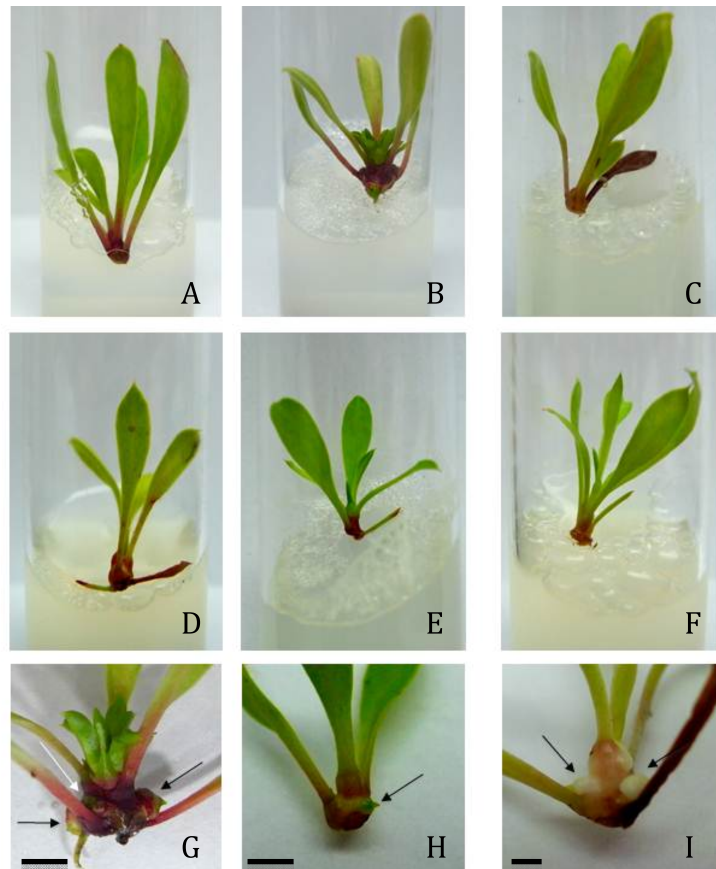

Fig. 3. Growth of microshoots under different light quality and media treatments, after 35 days in culture. (A) FL (0.1 mg L^{-1} NAA); (B) FL (2 mg L^{-1} zeatin, 0.5 mg L^{-1} IAA); (C) FR (0.1 mg L^{-1} NAA); (D) FR (2 mg L^{-1} zeatin, 0.5 mg L^{-1} IAA); (E) R+FR (0.1 mg L^{-1} NAA); (F) R+FR (2 mg L^{-1} zeatin, 0.5 mg L^{-1} IAA); (G) FL (2 mg L^{-1} zeatin, 0.5 mg L^{-1} IAA); (H) R+FR (0.1 mg L^{-1} NAA); (I) FR (2 mg L^{-1} zeatin, 0.5 mg L^{-1} IAA). FL = fluorescent lamp; FR = far-red light; R = red light. Arrow = new bud formation. Bar = 0.5 cm.