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Short note

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In vitro responses of Gerbera (*Gerbera jamesonii*) cultivars multiplied under different photoperiods

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Key words: cost reduction, *in vitro* development, light, micropropagation, shoot multiplication.

Abstract: Light affects several aspects of micropropagation. Five cultivars of gerbera were subjected to photoperiodic regimes of 10, 12 and 16-h of light for the shoot multiplication. The reduction of photoperiod from 16-h to 12-h resulted in increases in 41.8% to 97.2% of shoot multiplication in all gerbera cultivars.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

Gerbera is one of the most important species for cut flower market in the world, together with lilies, tulips and roses (Bhatia *et al*., 2011; University of Kentucky, 2015), owing to several breeding programs that have resulted in a rich diversity of colors (Cardoso and Teixeira da Silva, 2013).

Although gerbera can be propagated *in vivo* using seeds or rhizome division (Son *et al*., 2011), micropropagation using apical shoots or through organogenesis is the only way that can provide realistic large scale clonal propagation with genetic fidelity and disease free plantlets (Kanwar and Kumar, 2008; Bhatia *et al*., 2009) to meet the current demand of highly technological floriculture market.

Multiplication stage in micropropagation is one of the most crucial steps to establish *in vitro* propagation. In this regard, most of the earlier studies on gerbera micropropagation were focused on establishment of different types and concentrations of plant growth regulators.

Several factors affected gerbera *in vitro* shoot multiplication, as culture medium, plant growth regulators (especially citokinins as Benziladenine), growth conditions and the propagation method. However, genotype is the most important factor influencing *in vitro* development, including the rate of multiplication of gerbera (Son *et al*., 2011; Cardoso and Teixeira da Silva, 2013). The basal culture medium MS

(Murashige and Skoog, 1962) added 3% sucrose and Benziladenine (0.5 to 3.0 mg L^{-1}) is the most used for gerbera shoot multiplication (Kanwar and Kumar, 2008; Cardoso and Teixeira da Silva, 2013).

Although the chemical factors have been well studied in relation to the micropropagation for several species, including gerbera, the physical factors such as temperature and light (wavelength, PPFD and photoperiod) conditions are among the less discussed ones, despite their great biological and economic influence on the different development stages of micropropagation.

The electrical energy used in laboratories, especially in growth rooms, is the second most important cost factor of *in vitro* plantlet production, after labor cost (Cardoso and Teixeira da Silva, 2012). Therefore, an optimum light source is significant for this cost factor (Cardoso *et al*., 2013).

In this study, we tested different photoperiodic regimes at multiplication stage of micropropagation of gerbera cultivars to determine its effect on plant development and energy cost reduction in growth room conditions.

2. Materials and Methods

Five cultivars of gerbera were used in this study: 'Basic' and 'Kiserian' with both rose and pink inflorescences, and 'Orange Dino', 'Orca' and 'Onedin' with orange, white and yellow inflorescences, respectively.

The plant material used as donor was cultivated under greenhouse conditions and prepared as described by Cardoso and Teixeira da Silva (2012). Briefly, the plants in reproductive phase were completely defoliated and the rhizomes were maintained in cold chamber at 4°C for seven days, followed by transplanting these rhizomes to plastic pots containing organic substrate consisting of coconut fiber. After 14 days of transplantation, the first shoots were observed, and 21-28 days old shoots (5-10 cm in length and 2-3 leaves) were excised from the mother plants and were taken to the laboratory for surface disinfestations.

The shoots were reduced to 1.0-1.5 cm and washed for 5 min with tap water, followed by immersion in alcohol 75% (*v/v*), then in sodium hypochlorite with 1-1.25% of active chlorine for 20 min. Finally, the explants were washed three times in autoclaved distilled water. Shoot-tips of 2-3 mm length were used as explants to start gerbera micropropagation. The plants were multiplied to obtain the required quantity for the experiment.

The experiment was carried out during the multiplication stage of micropropagation. The culture medium used was Murashige and Skoog (1962) supplemented with 30 g L^{-1} of sucrose, 0.1 g L^{-1} of *myo*-inositol, 0.5 mg L^{-1} of benzyladenine (BA) and 6.0 g L^{-1} of agar-agar (Algagel, Type 900, Barueri, Brazil).

The flasks containing the culture medium and the individual shoots were maintained in growth room at 25±1°C and cultivated under cool white fluorescent lamps (Philips®, Barueri, Brazil) as light source with photon flux density of 20-25 µmol m^{-2} s^{-1}. The plants were subjected to three different photoperiods as follows: 10-h light/14-h dark, 12-h light/12-h dark and 16-h light/8-h dark. The last photoperiod condition was taken as control, as this photoperiod condition is regularly used for many species, including gerbera, for *in vitro* micropropagation (Cardoso and Teixeira da Silva, 2013).

In total, five borosilicate flasks (10 cm height x 5.5 cm diameter) containing four shoots, and covered with transparent polypropylene caps (repetitions), for each treatment were used. The experimental design was 5 × 3 factorial with five cultivars and three photoperiods.

The experiments were conducted for three months with time of transplanting of shoots each time for 30 days, resulting in three cultivation periods. In each pricking time, the multiplication factor was determined by counting the number of shoots obtained for the next subcultivation divided by the number of plantlets used at the start of the experiment. Five homogenized flasks with four plantlets in each turn were separated for the next evaluation, maintaining the plantlets in the same photoperiodic regime used in the previous experiment.

The data were subjected to the analysis of variance (ANOVA) and the Shapiro-Wilk test; if necessary, the data were normalized before comparison of means. The means were compared by the Scott-Knot test at 5% of probability.

3. Results, Discussion and Conclusions

Around 20% of the explants were successfully established *in vitro,* regenerated into plantlets, and transferred to the multiplication medium. Shoot apices with first leaf developed and without signals

of bacterial or fungal contamination in culture medium were transferred to the light condition and used to obtain the first shoots for the experiment. Around 60% of the explants were contaminated and around 20% formed callus with no regeneration into plantlets. The shoots so obtained were successfully *in vitro* multiplicated and used for the experiments of multiplication. These results were similar to those observed in other works at the establishment stage (Cardoso and Teixeira da Silva, 2012, 2013).

Genotype is one of the most important factors that affect the micropropagation, including the rate of multiplication and the responses to the factors affecting *in vitro* development of explants being micropropagated. This was also observed in our work with a positive interaction between the genotypes and photoperiods (Fig. 1). Under 16-h photoperiod, the cultivars 'Onedin', 'Orange Dino' and 'Orca' showed a higher multiplication rate (5.5, 5.0 and 4.8 shoots/explant, respectively) than the rose inflorescences cultivars Basic and Kiserian, which showed a lower multiplication rate of 3.6 and 2.4 shoots/explant, respectively. Interestingly, when the plantlets were subjected to the photoperiod of 12-h (4-h less than control photoperiod), only the cultivar Kiserian showed the multiplication rate less than 7.0 shoots/explant. Thus, it can be concluded that the response to the photoperiods has a direct correlation with the genotype used for micropropagation (Fig. 1). In fact, some cultivars of gerbera with rose and red inflorescences showed recalcitrance characteristics during *in vitro* multiplication, limiting their propagation and requiring protocol modification to accelerate the production of shoots and the efficiency of multiplication stage, compared with other cultivars. As example, a historical mean of multiplication along three years of *in vitro* cultivation showed that gerbera cv. Lamborghini (red inflorescences) was 3.8 shoots/explant compared with 6.8 and 4.6 shoots/explant for 'Suzzane' (orange) and 'Dino' (yellow) (Cardoso, personal observations in commercial lab).

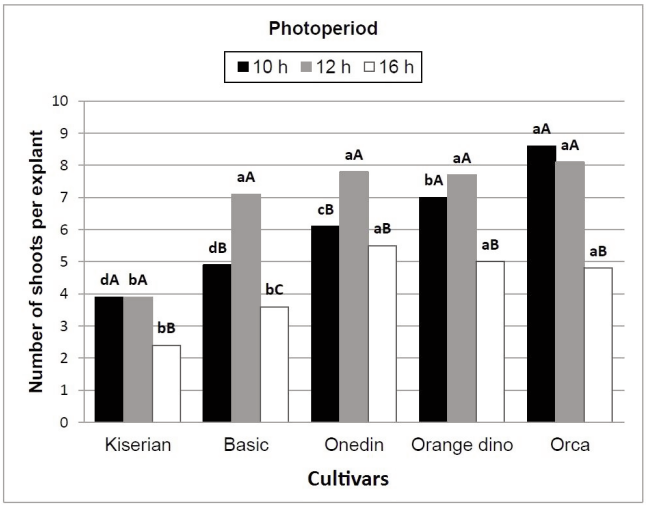

Fig. 1 - *In vitro* multiplication of gerbera (*Gerbera jamesonii*) cultivars under different photoperiodic regimes. The means were obtained from three multiplication times (experiments) considering five replicates (flasks) with four plants. Different small and capital letters show the differences among genotypes and photoperiod regimes, respectively, by the Scott-Knot test at 5% probability. The Coeficient of Variation is 13.52. The data were considered normal by Shapiro-Wilk normality test and the F was significative for all factors, including the interaction genotype x photoperiod.

The influence of genotype toward shoot multiplication was also observed by Hartl *et al*. (1993). They classified gerbera cultivars according to the rate of multiplication into three multiple-shoot induction cultivars: high (8 shoots/inoculum), moderate (6-7 shoots/inoculum) and low (4-5 shoots/inoculum). We also observed all these classes in our cultivars. However, the different photoperiodic conditions had different effects on the position of the cultivars in different classes of multiplication as proposed by Hartl *et al*. (1993). In our work the classification of cultivars using the Scott-Knot test with the best photoperiod for multiplication (12-h photoperiod) showed at least two classes of multiplication: (**a**) easily multiplied with number of shoots/explant >7.0; and (**b**) recalcitrant for multiple shoot induction, with the number of shoots/explant <4.0. An intermediary classes can also be considered as (**ab**), where the multiple shoot induction rates was 4 to 7 shoots/explant, but this was observed only in 10-h and 16-h photoperiod conditions.

The photoperiod had significant effects on gerbera *in vitro* multiplication (Fig. 1 and 2). Normally, the multiplication at 25±2°C associated with 16-h photoperiod is most used for gerbera (Cardoso and Teixeira da Silva, 2013), but in our actual work, we observed that for all five cultivars tested, the use of 12-h photoperiodic regime resulted in significant increase in multiple shoot induction when compared with 16-h standard photoperiod conditions (Fig. 1). The maximum positive effects (97.2%) were obtained in one of the most recalcitrant gerbera cultivar Basic, followed by 'Orca' (68.8%), 'Kiserian' (62.5%), 'Orange Dino' (54.0%) and 'Onedin' (41.8%).

Similar results were observed in micropropagation of ornamental pineapple *Annanas comosus* var. *erec-*

tifoilius, in which the reduction of photoperiod for 12-h resulted in better multiplication rate (6.6 shoots/explants) than 16-h photoperiod (4.2 shoots/explants) (Santos *et al*., 2015). The influence of photoperiod on *in vitro* development was also noted for other species and found affecting other *in vitro* plantlet characteristics of development, such as micro-tuberization in potato cultivars (Seabrook *et al*., 1993). In amaryllis (*Hippeastrum johnsonii*), the authors observed that 16-h photoperiodic regime resulted in better *in vitro* root length and number, bulblet diameter, and leaf length, compared with 14-h and 12-h of photoperiod (Zakizadeh *et al*., 2013).

Several amendments to reduce the cost have been proposed for gerbera micropropagation, such as chemical sterilization in place of autoclaving during all stages of micropropagation (Cardoso and Teixeira da Silva, 2012) and the use of greenhouse rooting and elongation, called PAG culture, in place of laboratory growth conditions (Cardoso *et al*., 2013). The reduction in photoperiodic regime resulted in low energy consumption and so the low cost for the multiplication stage of gerbera. This might be useful for the production of low cost plantlets from micropropagation of this important cut flower. The major conclusion can be drawn from this study is that a reduction in photoperiod by 4-h of light per day along with increase in multiplication rate may increase the efficiency of the propagation system. Chen (2016) concluded that the electrical energy for control environmental conditions on growth room, associated with the low efficiency of multiplication, is the most important factor that leads to increase cost of micropropagated plantlets of *Phalaenopsis*. This author showed that increase in multiplication rate from 1.5 to 2.5 could lead to a 50% cost reduction in micropropagated *Phalaenopsis*.

Photoperiod has a direct effect on the development of greenhouse cultivated gerbera genotypes, affecting plant width, height, shoot dry weight and number of flowers in a condition of 16-20-h of photoperiod (Gangnon and Dansereau, 1990). However, the interaction of photoperiod and the cultivation and development of gerbera could be more complex involving possible role of other environmental factors (Pettersen and Gislerød, 2003), and only few of them have been understood. Our results showed for the first time that the *in vitro* development of gerbera plantlets can be affected by photoperiod regimes (Fig. 1 and 2). Increases in axillary shoot production was observed in reduced photoperiods (12 and 10-h) compared with 16-h.

In addition, the use of white fluorescent lamps in the *in vitro* cultivation of gerbera resulted in a higher multiplication rate compared to different colors of light-emitting diodes (LEDs) lamps (Gök *et al*., 2016).

Our actual experiments showed that reducing the photoperiod to 12-h produced slightly increased shoot height with no visual effect on the size of leaves (Fig. 2). On the other hand, a further reduction of photoperiod to 10-h reduced the size of leaves (diameter and length) and resulted in etiolated development, showing negative effects on quality of gerbera shoots produced for rooting stage (Fig. 2). As example, the fresh weight of gerbera shoots in 10-h photoperiod is 20.0% ('Onedin'), 29.7% ('Orca') and 11.6% ('Orange Dino') less than compared with shoots produced in 12 or 16-h photoperiodic regimes (data not showed). Only gerbera cv. Kiserian showed practically the same fresh weight in 10-h and 12-h photoperiod.

Fig. 2 - Morphological characteristics of *in vitro* cultivation gerbera (*Gerbera jamesonii*) cv. Onedin under different photoperiodic regimes: 10-h and 12-h. Shoots from 10-h photoperiod presented smaller leaves (sl) and smaller and etiolated shoots (ses), compared to 12 and 16-h photoperiod. Bar = 1.0 cm

Plantlets obtained from all treatments were successfully *in vitro* rooted (95-100% of rooting) in ½MS with 30 g L^{-1} sucrose and 0.5 mg L^{-1} of indol-butyric acid and were acclimatized in greenhouse conditions using polypropylene trays with coconut powder as organic substrate. No morphological alterations were observed in acclimatized plantlets obtained, independent to the cultivar or photoperiod used, and the plantlets maintained the main characteristics of the donor plants until the flowering stage.

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