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RESEARCH ARTICLE

Growth Regulators Affected *In vitro* Propagation of Pot Gerbera (*Gerbera jamesonii* cv. Royal Soft Pink)

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ABSTRACT

Gerbera is known as an important plant in flower industry, and is considered as a favorite floricultural crop worldwide, ranked fourth among the top cut flowers. This research was conducted to investigate the effects of different concentrations of benzyladenine (BA) [1, 2, and 3 mgL^{-1}] solely and or in combinations with naphthalene acetic acid (NAA) [0.2 and 0.4 mgL^{-1}] on the induction, multiplication and proliferation of shoots in *Gerbera gamesonii* Bolus cv. Royal Soft Pink from shoot tip explants in a Murashige and Skoog (MS) medium. For rooting of shoots three concentrations of NAA (0, 0.5 and 1 mg L^{-1}) were examined. The results showed that the induction and proliferation of shoot were affected by the combination of BA and NAA. The highest number of shoots after two sub culture were obtained in MS medium supplemented with 2 mgL^{-1} BA and 0.2 mgL^{-1} NAA (10 shoots per explant). Considering root fresh and dry weights and root length in rooted plantlets it was not shown the significant difference between 0.5 and 1 mg L^{-1} NAA for rooting of shoots.

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INTRODUCTION

Gerbera (*Gerbera gamesonii* Bolus) belongs to Asteraceae family and its native distribution extends from Africa, to Madagascar, tropical Asia and South America (Bremer, 1994). This flower is one of the most important floricultural crops worldwide and usually grown as cut flowers, pot flowers, or garden plants. As a cut flower crop, it ranks fourth after roses, chrysanthemums, and tulips (AIPH, 2008) and generates sales valuated more than €100 million in just the Dutch auctions alone (Teeri *et al.,* 2006). Their great ornamental value is due to the typical capitulum inflorescence that displays a great variety of colors, and to the floral stem, which highly valued by consumers as individual vase decorations and bouquet compositions (Mata *et al.,* 2009). Gerbera can be conveniently propagated by both sexual (seed) and vegetative methods by using division of clumps or cutting the rhizomes (Cardoso and Teixeira da Silva, 2013; Minerva and Kumar, 2013). The high rate of variation from seed culture and a very low rate of multiplication through division could be considered as disadvantage of above mentioned methods. Therefore tissue culture method introduced a suitable propagation system with high rate of propagation and true to type production (Cardoso and Teixeira da Silva, 2013). Over the years, many scientists have performed the micropropagation of gerbera with different explants such as shoot tip, axillary bud, leaves, petiole, flower bud, capitulum, petal and ovule (Murashige *et al*., 1974; Shabanpour *et al.*, 2011; Cardoso and Teixeira da Silva, 2012). Murashige *et al*. (1974) reported that shoot tip technique is much more rapid, but the initial number of shoot tips required is very high due to the high infection rate (80%), and in turn require a great number of mother plants. Pierik *et al*. (1979) concluded that shoot tips are more suitable for mass propagation of gerbera in comparison to capitulum explant. Petru and Matous (1984) propagated shoot tips of gerbera on a MS medium containing 7 mg l^{-1} kinetin and 0.2 mg l^{-1} IAA. Aswath and Wazneen (2004) reported the effects of growth regulators and media on *in vitro* shoot regeneration and proliferation using shoot tips obtained

from ex vivo plants and showed the effectiveness of this system for commercial multiplication of gerbera. Gantait *et al*. (2010) used shoot tips excised from in vivo plants of *Gerbera gamesonii* 'Sciella' and cultured them on a medium containing 1.5 mg L^{-1} of BA and 0.5 mg L^{-1} NAA. They reported that this medium promoted earliest axillary bud initiation within 5 days and 91.6% of the explants and five axillary buds were initiated from a single explant within 13 days after culture. Cardoso and Teixeira da Silva (2012) used MS medium which was supplemented with 0.5 mg L^{-1} BA and 0.01 mg L^{-1} NAA for establishment of *in vitro* mother plants. For multiplication, the same medium was used except that 1.0 mg L^{-1} BA was applied and NAA was not added. The rooting and elongation medium consisted of 1/2 MS and 0.05 mg L^{-1} indole-3-butyric acid (IBA). Therefore, the aim of the present study was to develop a rapid and reliable protocol for *in vitro* shoot regeneration and rooting of shoots in pot flowering gerbera (*Gerbera jamesonii* cv. Royal Soft Pink).

MATERIALS AND METHODS

Culture condition and explant preparation

Seeds of commercial pot gerbera (*Gerbera jamesonii* cv. Royal Soft Pink) were kindly provided by Takii Europe BV (Netherlands). For surface sterilization, the seeds were dehusked and immersed in 70% alcohol for two min then in 5% commercial bleach (Clorox) for 5 min, and finally rinsed three times for 15 min in autoclaved distilled water. The seeds were dried with sterile filter paper and then were germinated aseptically on a MS basal medium (consisting of salts and vitamins) fortified with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar under light conditions (temperature: 23±1°C, relative humidity: 60% and photoperiod: 16-h) using white fluorescent lamps with a 60 μmol m^{-2} s^{-1} illumination. The pH of the MS medium was adjusted to 5.8 prior to autoclaving at 121°C for 20 min. The same MS medium and culture conditions were used for the other stages of micropropagation of this research. The seed germination occurred after 7 to 8 days and allowed the *in vitro* seedlings to grow for 20 days until the emergence of first true leaf. For the preparation of the explants, *in vitro* seedlings were transferred under a Laminar Air Flow Hood and shoot tips (4–5 mm of length) were separated and dissected to excise cotyledon leaves and true leaves.

Axillary bud induction, proliferation and rooting of shoots

The trimmed shoot tip explants were cultured on proliferation medium for supplemented with 1, 2 and 3 mgl^{-1} BA alone and in combination with 0.2 and 0.4 mgl^{-1} NAA (Table 1). The MS supplemented with 0.2 and 0.4 mgl^{-1} NAA and also MS without PGRs was evaluated in separate experiment. After two subculture which carried out at periodical interval of four weeks in this medium, the growth regulator-free MS medium was used for one week to synchronization of clumps. Individual shoots were transferred to MS medium containing 0.0, 0.5 or 1 mgl^{-1} NAA for root induction. Data that included the root length, root fresh and dry weight per plantlet, were recorded after 20 days of culture. For hardening, the plantlets with well-developed roots were removed from culture and washed in sterilized water to remove traces of the medium. These plantlets were transplanted to plastic pots containing a mixture of autoclaved cocopeat and perlite (1:1v/v) and maintained in a greenhouse for further adaptation and development. Visual quality was assessed by using a ranking scale of 1 to 10, 1= stunted, very compacted and having chlorosis in clumps; 10: normal growth, unstunted, not compacted and with clumps without chlorosis.

Statistical analysis

The experiments were based on completely randomized design with 6 replications (glass jars). Each replication was consist of 24 explants for bud proliferation and rooting respectively. Collected data in both experiments were statistically analyzed and the means were compared using Duncan's New Multiple Range Test (DNMRT) at 5% level of probability using the MSTATC program.

RESULTS

The effect of different concentrations of BA and NAA on the axillary bud formation

The results showed that the BA was crucial to bud proliferation of shoot tip explants. In separate experiment, in MS free hormone medium or media supplemented with NAA (0.2 and 0.4 mgl^{-1}) bud proliferation was not observed, while the size of one grown plantlet from shoot tip explant significantly increased and all of plantlets had the good appearance. In this experiment the highest number of leaves (13.5 leaves) per explant and shoot height (7.80 cm) were recorded in MS containing 0.4 mgL^{-1} NAA (Fig. 1). Moreover, root induction was occurred after 7-8 days of culture (data not shown) (Fig. 1).

In the main experiment the MS medium containing 2 mgL^{-1} BA and 0.2 mgL^{-1} NAA resulted the highest rate of proliferation after two subcultures (10 shoots per explant) (Table 1 and Fig. 2A). The lowest leaf number was observed in a MS supplemented with 1 mgL^{-1} BA and 0.2 mgL^{-1} NAA. The MS medium supplemented with 3 mgL^{-1} BA and 0.2 mgL^{-1} NAA had the highest leaf number per shoot although had not significant difference with most treatments. The lowest leaf number as well as the highest shoot height were obtained in MS medium containing 1 mgL^{-1} BA and 0.2 mgL^{-1} NAA. In all media the high quality of shoots were observed except in media containing high concentration BA (3 mg/l) which showed a stunt and hyperhydrated phenotype. The highest percentage of hyperhydrated shoots (32.33%) was obtained in a MS medium containing 3 mgL^{-1} BA alone. These shoots had abnormal growth with thick fragile and ridged leaves (Table 1 and Fig. 2B).

The effect of three concentrations of NAA on rooting of shoots

After 20 days of culturing shoots in free hormone MS medium or containing 0.5 and 1 mgL^{-1} NAA the parameters of root length, root fresh and dry weight per shoots were recorded. Although, root formation was

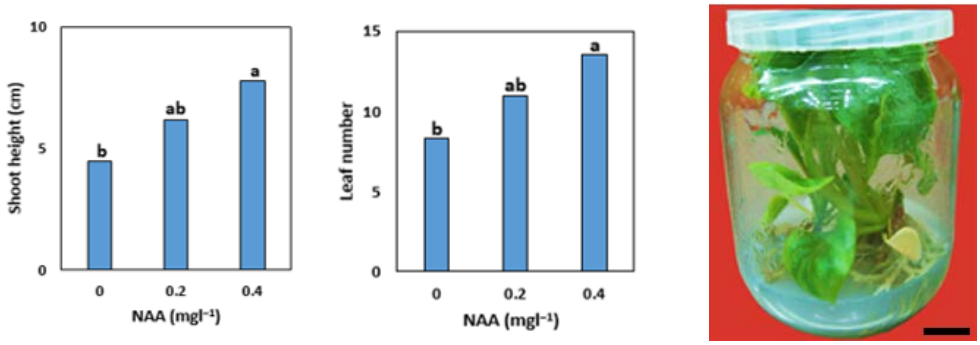

Fig. 1: The effect of three concentration of NAA (0, 0.2 and 0.4 mgl^{-1}) without BA on growth and development of shoot tip explant of *Gerbera jamesonii* cv. Royal Soft Pink (Left: plant height, middle: leaf number and right: growth and rooting of a shoot tip explant without any axillary bud formation in MS medium supplemented with only 0.4 mgl^{-1} NAA) (Scales bar: 1cm).

Table 1: Effects of different concentrations of BA alone and or in combination with NAA on shoot proliferation of *Gerbera jamesonii* cv. Royal Soft Pink.

PGRs (mgl^{-1})		No. of shoots per explant	No. of leaves per shoot	Shoot height (cm)	Shoot quality	Hyperhydracity (%)
BA	NAA					
1	0	5.00d*	4.70abc	3.51bcd	9.62a	1.00d
1	0.2	5.67cd	3.70c	5.10a	9.93a	0.83d
1	0.4	7.50bc	4.50bc	4.40ab	9.80a	0.50d
2	0	7.33bc	4.62abc	2.40d	9.93a	6.67c
2	0.2	10.00a	5.00abc	3.87bc	10.00a	3.00d
2	0.4	8.33ab	5.00abc	3.00cd	9.12a	3.00d
3	0	6.33cd	5.81ab	2.90cd	6.40b	32.33a
3	0.2	8.83ab	6.00a	3.42bcd	6.35b	24.33b
3	0.4	6.00cd	5.42ab	3.80bc	7.50b	25.00b

*In each column, means with the same letter (s) are not significantly different at 5% level of significance using DNMRT

obtained in all media, addition of NAA significantly increased the root numbers. However, no significant difference was observed among the 0.5 and 1 mgL^{-1} NAA. The MS supplemented with 0.5 mgL^{-1} NAA showed highest root length (3.45 cm), fresh (1.24 g) and dry weight (0.13 g) (Table 2 and Fig. 2C-D).

DISCUSSION

Growth and morphogenesis of *in vitro* plants are regulated by the interaction and a balance between the growth regulators supplied in the medium and the growth substances produced endogenously by the cultured cells (George and Sherrington, 1984). Regeneration in gerbera is depended on cultivar and PGR combinations, so the procedure of regeneration and media should be optimized for each cultivar (Shabanpour *et al*., 2011). The *in vitro* culture of shoot tip explants in gerbera is the most rapid form of clonal propagation and is one of the best methods to obtain plantlets from tissue culture without genetic and epigenetic changes that may occur through clonal somaclonal variation (Cardoso and Teixeira da Silva, 2013). In gerbera PGRs, mainly cytokinins are the most important factors affecting the shoot proliferation. Different combinations of cytokinins and auxins have been examined to achieve shoot induction and proliferation in gerbera. In the present study, among the different concentrations of BA that were used in combination with NAA, the MS medium supplemented with 2 mgl^{-1} BA+0.2 mgl^{-1} NAA showed significantly superiority compared to others because the higher number of plantlets with good quality that were obtained. Similarly, Gantait *et al*. (2010) obtained highest multiplication rates in cultivar 'Sciella' of gerbera on MS medium containing 1.5 mg L^{-1} BA and 0.5 mg L^{-1} NAA. In another study, most multiple shoots were produced in full-strength MS medium with 500 mg L^{-1} casein hydrolysate, 1.0 mg L^{-1} BA and 0.2 mg L^{-1} NAA. Son *et al*. (2011) obtained a ranging between 5.8 and 15.5 shoot multiplication rate in MS with 3.0 mg L^{-1} BA and 0.1 mg L^{-1} NAA in four gerbera cultivars. Moreover, previous studies have shown that the most multiplication rates of shoots per explant were obtained in MS medium supplemented with 2.0 mg L^{-1} BA and 0.3 mg L^{-1} NAA (Feng *et al*. 2009). This difference highlights the importance of exogenous supply of growth regulators to achieve higher multiplication rates. However, optimum concentration of growth regulators varies with different cultivars. More ever to purpose of propagation by shoot tip culture explant an additional advantage of this method is the genetic stability inherent because the new shoots originated from apical meristem and propagation from adventitious meristems can be avoided (Grout and Brian, 1999). Constantinovici and Sandu (1995) reported that the rooting of gerbera is easy and possible in media without PGRs, and concluded that plants that rooted *in vitro* showed better acclimatization than unrooted ones. In this study all of plantlets were rooted, even in NAA-free MS medium, but significant difference was found between this treatment and MS supplemented with 0.5 and 1 mgL^{-1} NAA treatments. The best rooted plantlets were produced using a rooting medium containing MS medium and 0.5 mgL^{1} NAA. Sahavacharin (1985) indicated that the best induction of adventitious roots in gerbera was in MS supplemented with 0.5 mg L^{-1} IAA. In general, a number of researchers examined variou auxins (IAA, NAA and

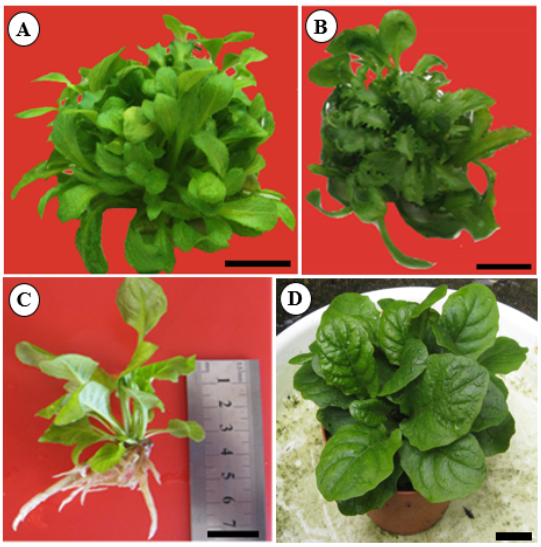

Fig. 2: Micropropagation of *Gerbera jamesonii* cv. Royal Soft Pink by shoot tip explants. (A) Axillary bud formation and multiple shoot proliferation from a shoot tip in MS medium containing 2 mgl^{-1} BA and 0.2 mgl^{-1} NAA; (B) abnormal shoot growth with thick fragile and ridged leaves which were also vitrificated in MS medium supplemented with only 3 mgl^{-1} BA; (C) an individual plantlet rooted *in vitro* after removing from of glass jar and washing MS medium and (D) Five month old acclimatized plant growing in greenhouse (Scales bar: 1cm).

Table 2: Effects of NAA on root parameters of *Gerbera jamesonii* cv. Royal Soft Pink shoots

NAA (mgl^{-1})	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
0	1.03b	0.84b	0.07b
0.5	3.45a	1.24a	0.13a
1	3.52a	1.39a	0.13a

*In each column, means with the same letter(s) are not significantly different at 5% level of significance using DNMRT

IBA) with different concentrations for rapid rooting, best rooting percentage and highest number of adventitious roots per *in vitro* regenerated shoots in gerbera (Gantait *et al.*, 2010; Shabanpour *et al.*, 2011; Cardoso and Teixeira da Silva, 2012; Shabbir *et al.*, 2012; Naz *et al.*, 2012). Vitrification, a common problem in gerbera tissue culture, caused morphological changes of regenerated shoots during *in vitro* micropropagation. The results of present experiment indicated that vitrified shoots were short and thick with fragile and breakable leaves and the highest percentage of vitrified plantlets was obtained in MS medium containing only 3 mgL^{-1} BA. However, decreasing the concentration of BA from 3 to 2 mg L^{-1}, decreased the rate of vitrification (Table 1). Based on the results of this study, it can be concluded that BA stimulates shoot proliferation in gerbera, but at the same time, especially at higher concentrations, increases the rate of vitrification. Consequently, it may be suggested that for obtaining normal plantlets with a minimum of vitrification rate lower concentration of BA (2 mg L^{-1}) should be used (Table 1). Sharma and Mohan (2006) indicated that using of BA in high concentrations on the MS medium caused the vitrification phenomenon in *Chlorophytum borivilianum*. Feng *et al*. (2009) reported that only concentrations higher than 3 mg L^{-1} of BA increased hyperhydricity in gerbera seedlings cultivated *in vitro* and also observed that a high concentration of NAA (higher than 0.3 mg L^{-1}) resulted in chlorotic leaves in micropropagated plants, showing a genotype-dependence of *in vitro* hyperhydricity and other abnormalities. In *Gerbera aurantiaca* the highest concentration of BA (2.25 mg L^{-1}) resulted in 20% hyperhydric shoots (Meyer and Van Staden, 1988). It may be conclude that the response of gerbera to tissue culture is almostly genotype dependent and for this reason, selection of cultivar is one of the main factors affecting the regeneration and multiplication of gerbera shoots (Cardoso and Teixeira da Silva, 2013). Direct shoot regeneration using shoot tips as initial explant is most convenient method for mass propagation of gerbera (Minerva and Kumar, 2013).

In conclusion, we have developed an efficient method to promote axillary bud proliferation from shoot tip explants followed by high frequency of rooting plantlets for *Gerbera jamesonii* cv. Royal Soft Pink. These results can be used for the ornamental plant propagators and producers of plug transplants.

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