African Journal of Biotechnology Vol. 8 (9), pp. 1860-1863, 4 May, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Regeneration of begonia plantlets by direct organogenesis

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Accepted 19 February, 2009

The economic importance of ornamentals worldwide suggests a bright future for ornamental breeding. Rapid progress in plant molecular biology has great potentials to contribute to the breeding of novel ornamental plants utilizing recombinant DNA technology. The plant cell, tissue or organ culture of many ornamental species and their regeneration are essential for providing the material and systems for their genetic manipulation, and this is therefore the first requirement of genetic engineering. In this research, different concentration of BA (0.0, 0.5, 1.0, 2.0 mgl^{-1} with NAA (0.0, 0.5, 1.0 mgl^{-1}) and BA (0.0, 0.5, 1.0, 2.0 mgl^{-1}) with IAA (0.0, 0.5, 1.0, mgl^{-1}) were investigated to optimize regeneration of *Begonia elatior* cv. Toran orange. The best regeneration and growth were obtained from the media containing 2.0 mgl^{-1} BA and 1.0 mgl^{-1} NAA (70%) followed by 1.0 mgl^{-1} BA and 0.5 mgl^{-1} NAA (50%), 1.0 mgl^{-1} BA and 1.0 mgl^{-1} NAA (20%) in BA - NAA combination. The media with BA - IAA combination showed that the best regeneration was 0.5 mgl^{-1} BA and 0.5 mgl^{-1} IAA (43%) followed by 0.5 mgl^{-1} BA and 1.0 mgl^{-1} IAA (23%).

Key words: Begonia, *Begonia tuberus*, ornamental, regeneration, direct organogenesis.

INTRODUCTION

World trade in the floriculture industry was estimated at approximately $44 billion with the Netherlands, Japan, and the United States (US) leading global production in 2002 (Marques and Caixeta, 2003). The consumption of floriculture products per capita varies greatly in different countries. A recent review (Chen et al., 2005) highlights the production conditions needed for the foliage plant industry. Compared to other (edible) horticultural crops, floricultural products need more investment, a higher growing technology and more precise management. Therefore profit in floricultural products per unit area is much higher than that of other agricultural products (Xia et al., 2006). Floriculture is divided into four groups: cut flowers, cut foliage, pot plants and bedding/garden plants.

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Abbreviations: BA, 6-Benzyladenine; **IAA**, indole-3-aceticacid; **NAA**, 1-naphthyleneacetic acid.

Begonia, a genus in the flowering plants family Begoniaceae is one of the ten largest angiosperm genera. Begonias are attractive perennial herbs with soft, succulent stems, and white, pink, red, orange, or yellow flowers. The begonia family consists of 5 genera and 920 true species, majority of which belong to the genus Begonia. Begonia's taxonomy can be ambiguous, mainly due to the enormous number of horticultural varieties and hybrids, which many gardeners treat as species. These horticultural varieties of begonia number in their thousands. This is a large genus, and countless hybrids have been introduced, which largely fall into 8 distinct groups: cane-like, rex-cultorum, rhizomatous, semperflorens, shrub-like, thick-stemmed, trailing or scandent and tuberous (Gardening Australia, 2008).

The most common types of begonias for growing outside are the fibrous rooted or wax and tuberous begonias. Unlike their semperflorens cousins, tuberous begonias offer wide color choices: white, pink, rose, red, orange and yellow. Both the large and small flowered tuberous begonias alternatively bear male (ravishingly beautiful) and female (single and smaller) flowers. New begonia plants are reproduced from seed, tubers or cutt-

ings. Plant breeding has produced many showy flower varieties. Most begonias can be grown outdoors year-round in subtropical or tropical climates, but in temperate climates begonias are grown outdoors as annuals or as house or greenhouse plants but tuberous begonias usually have a dormant period, during which the tubers can be stored in a cool and dry place. Most begonias are easily propagated by division or from stem cuttings. In addition, many can be propagated from leaf cuttings or even sections of leaves, particularly the members of the rhizomatous and rex groups.

Recent modern techniques of propagation have been developed which could help growers to meet the demand of the ornamental industry in the next century. An overview on the *in vitro* propagation via thin cell layer, meristem culture, regeneration via organogenesis and somatic embryogenesis is presented. Available methods for the transfer of genes could significantly simplify the breeding procedures and overcome some of the agronomic and environmental problems, which other wise would not be achievable through conventional propagation methods. The development and remarkable achievements with biotechnology in ornamental pot plants made during the 3 decades have been reviewed (Rout et al., 2006).

In vitro regeneration, transformation and micropropagation of begonia via organogenesis, tissue culture and transverse thin cell layer technology were investigated by different researchers (Takayama and Misawa,1982; Bowes and Curtis, 1991; Nakano et al., 1999; Bouman and Klerk, 2001; Kishimoto et al., 2002; Burritt et al., 2003; Acquaah, 2004; Espino et al., 2004; Nhut et al., 2005; Shimada et al., 2006, 2007). In this research, the regeneration of *Begonia tuberus* was optimized using different hormone concentrations and explant types for future transformation and breeding studies.

MATERIALS AND METHODS

The experiment was conducted in the Laboratory of Tissue Culture and Biothecnology, Horticulture Department, Agriculture Faculty, Cukurova University, Adana, Turkey. In this research, potted *Begonia elatior* cv. Toran orange plants obtained from a commercial company (Fidan Seracılık) were washed for 30 min under running tap water, then incubated in 70% ethanol for a few seconds and 20% NaOCl (natrium hypochloride) containing few drops of tween-20 for 20 min. They were rinsed in sterile water, 4 - 5 times, sterilized and placed on an MS basal medium (Murashige and Skoog, 1962). The medium was adjusted to pH 5.7, was autoclaved at 1.05 kg/cm² and 121°C for 20 min.

Leaf (0.5 × 0.5 cm) and pedicel (5 mm long) segments of begonia plants were used as explants. The experiment was set as 5 replicates for each of the 12 concentrations containing 6 explants. Leaf and pedicel explants were cut in the sterile cabinets and placed in the MS medium containing different concentrations and combinations of BA (0.0, 0.5, 1.0, 2.0 mgl^{-1}) with IAA (0.0, 0.5, 1.0 mgl^{-1}) and BA (0.0, 0.5, 1.0, 2.0 mgl^{-1}) with NAA (0.0, 0.5, 1.0 mgl^{-1}). All explants were incubated at 25 ± 2°C with 16/8 h light/dark photoperiod by white flouresent light (3000 – 4000 lux).

RESULTS AND DISCUSSION

Observations were obtained 6 weeks after the experiment was set up and findings are shown in Figures 1 and 2. As it can be seen from Figure 1, the best regeneration started from pedicel explants placed on the media containing 2 mgl^{-1} BA – 1 mgl^{-1} NAA (70%) followed by 1.0 mgl^{-1} BA - 0.5 mgl^{-1} NAA (50%) and 1 mgl^{-1} BA – 1 mgl^{-1} NAA (20%). Although the medium with 2 mgl^{-1} BA - 0.5 mgl^{-1} NAA produced maximum regeneration ratio at the first stage, further proliferation was not succesfully obtained.

High cytokinin concentration combined with auxin was more effective than cytokinin used alone on the regeneration of begonia. The reason for low regeneration of the medium containing 1.0 mgl^{-1} BA - 1.0 mgl^{-1} NAA could be the antagonistic effect of high NAA on cytokinin. Pierik (1997) mentioned that cytokinins are often used to stimulate growth and development. They usually promote cell division, especially if added together with an auxin. *In vitro* regeneration of 4 Begonia genotypes, *Begonia semperflorens*, *Begonia rex, B. elatior*, and a hybrid of Begonia with unknown parents 'Tiger' was carried out starting from leaf and petiole segments as explants by Espino et al. (2004). Five Murashige and Skoog's derived media were tested, 3 of them supplemented with alpha-naphthaleneacetic acid (NAA) and 6-benzyladenine (BA), and the other 2 with NAA and kinetin (KIN) in different concentrations. It was mentioned that shoot regeneration was preferentially induced on the BA containing media, quantitative differences being observed among explants and genotypes. Takayama and Misawa (1982) reported that the medium containing 1.3 µM BA or 4.6 µM Kn along with 5.4 µM NAA showed rapid regeneration of shoot buds from leaf and petiole segments of the begonia species.

Comparism of the media containing 1.0 mgl^{-1} BA - 0.5 mgl^{-1} NAA (50 %) and 1.0 mgl^{-1} BA -1.0 mgl^{-1} NAA (20%) showed that increasing auxin concentration decreased the regeneration ratio although the cytokinin concentration was same for each of them (Figure 1). NAA is known as a strong auxin, so it eliminated the regeneration effect of BA in the media containing 0.5 mgl^{-1} BA - 0.5 mgl^{-1} NAA and 0.5 mgl^{-1} BA - 1 mgl^{-1} NAA. Pierik (1997) also mentioned that auxins generally cause cell elongation, swelling of tissues, callus formation and especially inhibition of adventitious and axillary shoot formation.

The same situation was shown for BA and IAA combination if the media containing 0.5 mgl^{-1} BA - 0.5 mgl^{-1} IAA (43 %) and 0.5 mgl^{-1} BA - 1 mgl^{-1} IAA (23 %) were compared (Figure 2). Higher auxin caused lower regeneration whenever cytokinin concentration was kept constant in the media. Higher BA concentration with IAA (2.0 mgl^{-1} BA - 1.0 mgl^{-1} IAA) showed very low regeneration at 3.0% ratio. These results are similar with the results obtained by Nhut et al. (2005). The highest shoot

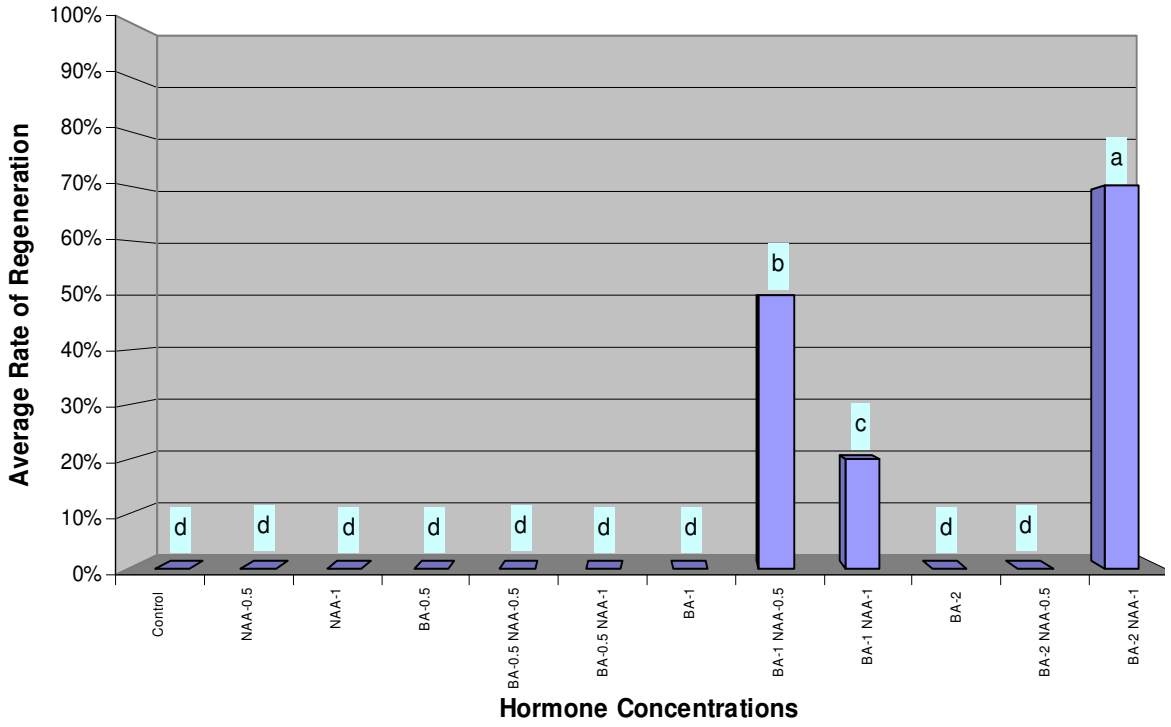

Figure 1. Regeneration of *B. tuberus'* pedicel explant in BA-NAA hormone concentrations. A triangilation approach was adopted and both multiple comparisons tests of Turkey's HSD and Fisher's least significiant difference (LSD) were run to identify whether regeneration in different hormone concentrations are significiantly different at 0.05 level. In the figure, mean with the same letter are not significantly different according to LSD and Turkey's HSD tests at 0.05.

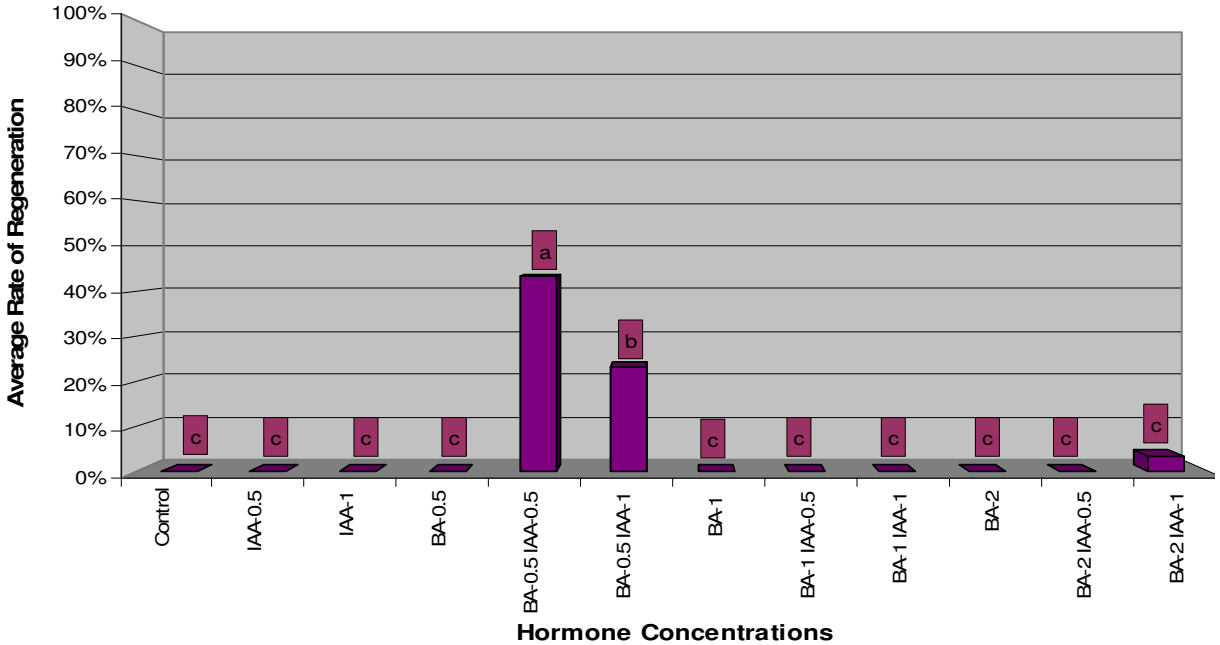

Figure 2. Regeneration of *B. tuberus'* pedicel explant in BA-IAA hormone concentrations. A triangilation approach was adopted and both multiple comparisons tests of Turkey's HSD and Fisher's least significiant difference (LSD) were run to identify whether regeneration in different hormone concentrations are significiantly different at 0.05 level. In the figure, mean with the same letter are not significantly different according to LSD and Turkey's HSD tests at 0.05.

formation (56.67 %) obtained from caulogenesis was recorded in the presence of 1.0 mgl^{-1} BA alone. When both BA and auxin were used at low concentrations, shoot formation was over 75%. In combination, shoot formation rate was recorded to decrease as BA concentration was increased.

The results showed that the combination of BA and NAA gave better cell division and regeneration than the combination of BA and IAA for pedicel explants. There was no regeneration from the media containing cytokinin or auxin alone. Leaf tissue was also used as an explant. Although statistical data (not shown here), indicates that there was no shoot regeneration from the leaf explants but some callus formation.

ACKNOWLEDGEMENT

This study was supported by Scientific Research Project in Cukurova University (ZF 2007BAP23).

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