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BRIEF COMMUNICATION

# Shoot regeneration in four *Begonia* genotypes

F.J. ESPINO, R. LINACERO, J. RUEDA and A.M. VÁZQUEZ*

*Departamento de Genética, Facultad de Biología, Universidad Complutense de Madrid, E-28040 Madrid, Spain*

## Abstract

*In vitro* regeneration of four *Begonia* genotypes, *B. semperflorens*, *B. rex*, *B.* × *elatior*, and hybrid of *Begonia* with unknown parents 'Tiger' was carried out starting from leaf and petiole segments as explants. Five Murashige and Skoog's derived media were tested, three of them supplemented with α-naphthaleneacetic acid (NAA) and 6-benzyladenine (BA), and the other two with NAA and kinetin (KIN) in different concentrations. Shoot regeneration was preferentially induced on the BA containing media, quantitative differences being observed among explants and genotypes.

*Additional key words*: explant type, genotype effect, growth regulators, *in vitro* culture, organogenic response.

*Begonia* genus contains around 2000 species, of which over 200 have been introduced into the market by commercial growers. Some species such as *Begonia semperflorens* and *B. rex*, and hybrids such as 'Tiger' with unknown parents, *B. tuberhybrida*, *B.* × *elatior* (= *B.* × *hiemalis*) and *B.* × *erythrophylla* are among the most important ornamental plants. These plants are usually propagated by conventional vegetative methods, such as stem and leaf cuttings, although some of them can also be reproduced by seeds, as in the case of *B. semperflorens.* The vegetative propagation of begonias has been improved by the application of plant tissue culture techniques (Takayama 1990). Although the tissue culture methods and conditions are similar for different begonias, their requirements of growth regulators in the culture media are different. The results obtained by several authors (Chlyah and Tran Thanh Van 1975, Khoder *et al.* 1981, Simmonds 1984, Cassells and Morrish 1987, Burrit and Leung 1996, Kiyokawa *et al.* 1996) show that diverse auxin and cytokinin concentrations and/or their combinations are required in different species. So, the development of a good regenerative system for each genotype is important, mainly if genetic transformation experiments are to be applied. In this respect, Kiyokawa *et al.* (1996) reported the first results on *Begonia tuberhybrida* transformation, based on a comparative organogenetic study among three *Begonia* species.

In the present work, four begonias, three types of explants and five culture media have been tested in order to ascertain the morphogenetic response of these genotypes under the same *in vitro* conditions and to verify if those protocols could be useful to establish an efficient regeneration method to be applied in further transformation experiments.

The four *Begonia* genotypes were *B. semperflorens* cv. Thousand Wonders, *B. rex* Putz. cv. Lucille Closon, *B.* cv. Tiger and *B.* × *elatior* cv. Schwabenland orange. The *B. semperflorens* donor plants were obtained from *in vitro* germinated seeds and in the other cases the donor plants were obtained from a local grower.

The *B. semperflorens* seeds were surface sterilised (1 min in 70 % ethanol and then 20 min in 1.5 % m/v sodium hypochlorite solution), rinsed three times in sterile distilled water and placed on MS medium (Murashige and Skoog 1962) supplemented with 3 % sucrose and 0.8 % agar. The pH was adjusted to 5.8 before autoclaving. The cultures were maintained in a

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*Abbreviations*: BA - 6-benzyladenine, KIN - kinetin, MS - Murashige and Skoog; NAA - α-naphthaleneacetic acid.

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* Author to whom correspondence should be addressed; fax: (+34) 913 944 844, e-mail: anavaz@bio.ucm.es

growth chamber at 25 ºC under a 16-h photoperiod (irradiance of 42 μmol $m^{-2}$ $s^{-1}$, *Silvania Gro-Lux* and *Philips* cool white light tubes) until plantlets developed.

Three explants were used: leaf squares of 0.25 and 1 $cm^2$ and petiole sections of 2 mm. The explants of *B. semperflorens* were taken from one month old *in vitro* developed plantlets. For the other three genotypes, leaves with their petioles were taken from mature plants and surface sterilised following the already mentioned protocol for seeds.

The explants were cultured on five MS derived media supplemented with different concentrations and combinations of growth regulators: 1 mg $dm^{-3}$ NAA and 1 mg $dm^{-3}$ BA (NB medium), 0.1 mg $dm^{-3}$ NAA and 0.5 mg $dm^{-3}$ BA (N1B5 medium), 0.1 mg $dm^{-3}$ NAA and 0.1 mg $dm^{-3}$ BA (N1B1 medium), 0.01 mg $dm^{-3}$ NAA and 0.1 mg $dm^{-3}$ KIN (N01K1 medium), and 0.1 mg $dm^{-3}$ NAA and 0.1 mg $dm^{-3}$ KIN (N1K1 medium). All media contained 3 % sucrose and 0.8 % agar and the pH was adjusted to 5.8 before autoclaving. The culture conditions were the same ones described for seed germination. The explants were cultivated for 12 weeks and morphogenetic response was evaluated with the stereomicroscope (*Stemi SR*, *Zeiss*, Jena, Germany).

The morphogenetic response observed 6 weeks after the initiation of the cultures was the formation of buds directly on the explant surface which, later, developed into shoots. After 12 weeks in culture, the number of regenerated shoots was scored.

In the BA containing media, the four genotypes were able to regenerate shoots. The response in the cultures initiated from the 0.25 $cm^2$ explants was higher than in those initiated from the 1 $cm^2$ explants in all the cases except in the *B.* × *elatior* cultures, in which the same amount of regenerated shoots per $cm^2$ was found (data not shown). This is in agreement with the observation that in all but the *B.* × *elatior* cultures regeneration started in the cut edges of the leaf explants.

However, the four genotypes differed in their ability to regenerate in the BA containing media. *B. rex* was the most responsive, its worst results, in N1B1 medium, being better than those of *B.* × *elatior* and *B. semperflorens* in their best media, NB and N1B5 respectively (Table 1). These two genotypes showed the lowest regeneration rate in our conditions. In *B.* × *elatior*, also the addition of 0.5 mg $dm^{-3}$ of indole-3-acetic acid (IAA) and 1 mg $dm^{-3}$ of BA to the regeneration medium and the use of young tissues as explants do not seem to improve regeneration (Kishimoto *et al.* 2002).

Effect of BA concentration was clearly detected in the *B. rex* leaf cultures, since the amount of responsive explants and the number of shoots per cultured explant decreased as the BA concentration decreased. This effect was also described in *Cardiospermum halicacabum* cultures (Babber *et al.* 2001) in which the regeneration rate was optimal with 4 mg $dm^{-3}$ of BA. The opposite BA dose effect was found in the *B.* 'Tiger' cultures. NB medium failed to induce shoot regeneration, but it was efficient in the N1B1 medium and less markedly in the N1B5 one. A negative effect of the high concentration of NAA in the NB medium, compared to N1B1 and N1B5 media, could also account for preventing regeneration of this genotype.

Table 1. Shoot regeneration from 0.25 $cm^2$ leaf explants. The culture medium and genotype combinations which do not appear in the table did not regenerate shoots (means ± SD).

| Genotype | Medium | Number of explants | Responsive explants [%] | Number of shoots [explant$^{-1}$] |
|---|---|---|---|---|
| *B.* × *elatior* | NB | 64 | 48.44 | 3.73 ± 0.75 |
| *B. semperflorens* | NB | 30 | 56.70 | 1.73 ± 0.33 |
| | N1B5 | 20 | 70.00 | 2.25 ± 0.46 |
| *B. rex* | NB | 66 | 100.00 | 41.90 ± 1.33 |
| | N1B5 | 76 | 93.42 | 20.20 ± 0.77 |
| | N1B1 | 50 | 62.00 | 3.94 ± 0.86 |
| *B.* 'Tiger' | N1B5 | 15 | 40.00 | 1.40 ± 0.68 |
| | N1B1 | 14 | 64.28 | 8.21 ± 2.26 |

The media supplemented with KIN failed to induce shoot regeneration in the four genotypes. In these media, the explants turned brown within a few days after the initiation of the culture and died. Although Khoder *et al.* (1981) reported that KIN can efficiently induce bud regeneration from different explants in *B.* × *elatior*, it was when the culture medium was supplemented with 2 mg $dm^{-3}$ of KIN. So, these different results could be due to different concentrations of KIN used in each case.

The regeneration rates of *B.* × *elatior*, *B. semperflorens* and *B.* 'Tiger' in petiole cultures was higher than that in leaf explant cultures in the same genotypes, only *B. rex* petioles showing a lower response in the three media in which regeneration took place (Table 2).

Although in *B. semperflorens* and *B. rex* a few shoots developed, again, in the majority of the cultures, regeneration from petiole explants was not induced in the KIN containing media. As mentioned above, it is possible that higher KIN concentrations could improve the morphogenetic response (Khoder *et al.* 1981).

In *B.* × *elatior* the responsive petioles on NB medium developed numerous buds which completely covered the explant. However, these buds did not develop at all into shoots and degenerated after some time in culture. In N1B5 medium the regenerated shoots did not develop properly either, but in N1B1 medium their development was correct. Simmonds (1984) reported the same behaviour in relation to induction and growth of

adventitious shoots in culture media which were equivalent to our N1B5 and N1B1 from petiole explants of *B.* × *hiemalis* (= *B.* × *elatior*).

Table 2. Shoot regeneration from petiole explants. The culture medium and genotype combinations which do not appear in the table did not regenerate shoots (means ± SD).

| Genotype | Medium | Number of explants | Responsive explants [%] | Number of shoots [explant$^{-1}$] |
|---|---|---|---|---|
| *B.* × *elatior* | NB | 114 | 11.40 | not scored |
| | N1B5 | 42 | 16.67 | 7.40 ± 2.72 |
| | N1B1 | 69 | 13.04 | 2.45 ± 0.83 |
| *B. semperflorens* | NB | 35 | 94.29 | 11.29 ± 1.00 |
| | N1B5 | 20 | 100.00 | 16.00 ± 1.34 |
| | N1B1 | 40 | 92.50 | 7.48 ± 0.67 |
| | N01K1 | 25 | 4.00 | 0.08 ± 0.08 |
| | N1K1 | 62 | 19.35 | 0.24 ± 0.07 |
| *B. rex* | N1B5 | 70 | 20.00 | 1.35 ± 0.39 |
| | N1B1 | 72 | 66.67 | 2.73 ± 0.54 |
| | N01K1 | 90 | 68.89 | 1.34 ± 0.19 |
| *B.* 'Tiger' | N1B5 | 41 | 82.92 | 6.83 ± 0.75 |
| | N1B1 | 20 | 100.00 | 14.40 ± 1.39 |

*B. semperflorens* petiole cultures showed a very good regeneration rate in the BA containing media, being significantly higher in the N1B5 medium than in the NB and N1B1 media. The petioles proved to be a more regenerative explant than the leaves in these conditions. These results are in agreement with those of Kiyokawa *et al*. (1996), although these authors also reported an efficient regeneration from leaf explants on a medium similar to N1B1, while in our case no regeneration was observed. The N1B5 medium was also the best one for shoot regeneration in cultures of *Spilanthes acmella* Murr. (Saritha *et al.* 2002).

In *B. rex*, regeneration from petiole explants was very low, the N1B1 medium showing the best response. Cassells and Morrish (1987) also reported shoot regeneration in *B. rex* using petioles or leaves with a BA concentration lower than the one in our N1B1 medium. In the KIN supplemented media, petiole explants yielded shoot regeneration only when cultured on N01K1 medium, the number of shoots per cultured explant being similar to N1B5 medium.

The petiole explants of *B.* 'Tiger' regenerated only when cultured on N1B1 and N1B5 media. Again a BA concentration effect was detected that affected the regeneration. BA concentration and regeneration ability were inversely related and, in this genotype, leaf explants behaved in a similar way. Shoot regeneration was completely abolished at the highest BA concentration (NB medium) and the frequency of regenerated shoots per cultured explant and the percentage of responding explants increased as the BA concentration was lowered, reaching a maximum on N1B1 medium. The high concentration of NAA in NB medium could also act negatively upon the shoot regeneration process. The data described here are the first ones reported on *in vitro* regeneration of *B.*'Tiger'.

In all the cases in which shoots were regenerated, the recovery of the complete plants was very easy: after rooting in a MS hormone free medium the plantlets were transferred to *in vivo* conditions with a high rate of survival.

We can conclude that shoot regeneration was genotype and explant dependent and it was very efficient using petiole segments of *B. semperflorens* on N1B5 medium, 0.25 cm$^2$ leaf explants of *B. rex* on NB medium, and petiole segments of *B.* 'Tiger' on N1B1 medium. Although *B.* × *elatior* showed a lower regeneration response than the other genotypes, leaf explants on NB medium and petiole explants on N1B1 medium gave the best response in our conditions.

Preliminary data indicate the possibility of recovering transgenic plants of *B.* 'Tiger' using the above indicated regeneration procedure and the *Agrobacterium tumefaciens* transformation method.

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