

IN VITRO REACTIVITY OF *Begonia semperflorens* cv. ‘Ambassador’ White TO GROWTH REGULATORS

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Abstract. The objective of this experiment was to investigate the regeneration potential of a *Begonia semperflorens* cv. ‘Ambassador’ White cultivated on Murashige-Skoog standard mineral medium, supplemented with different combinations of growth regulators. After 90 days of *in vitro* culture, multiple shoots with 13.3 mm diameter with 12.5 leaves over 6 mm in diameter and with 4-5 buds, were efficiently obtained from the subcultured small propagules on variant V₂ - mineral basic medium culture MB - MS supplemented with 1.5 mg / l thidiazuron. *Callus* induction was favored only on experimental variant V₃ - mineral basic medium culture MB - MS supplemented with a mix of 1.5 mg / l 3-Indolebutyric acid plus 1.5 mg / l thidiazuron, with highest diameter of *callus* of 9 mm.

Keywords: *Begonia semperflorens*, subcultivation, cytokine; auxine, micropropagation.

INTRODUCTION

Micropropagation method is applied to various species of *Begonia*, which lists some research with their successful multiplication *in vitro*, such as: *Begonia cheimanthus* [5, 6, 16], *Begonia erythrophylla* [21], *Begonia rex* [2], *Begonia tuberhybrida* [11, 14, 26, 30], *Begonia richmondensis* [20], *Begonia evansiana*, *Begonia sutherlandii*, *Begonia socotrana* [19], *Begonia franconis* [3], *Begonia venosa* [15] etc.

In the case of *Begonia* sp. *micropropagation* it has been applied a method, in which buds give rise to plantlets in a short time and without difficulties. In few researches conducted by Takayama and Misawa, Murashige-Skoog medium was most effective in micropropagation of several hybrids or *Begonia* species like *Begonia x hiemalis* and *Begonia tuberhybrida* [26].

Generally, regeneration and differentiation of adventitious shoots is promoted by cytokines. Once the pathway is established by hormonal control the regenerative tissue will follow the morphogenetic pathway even in the absence of growth regulators [4, 27]. This strategy was successful to initiate *in vitro* cultures of *Begonia*, from different explants of plant material [16, 30]. This method was also effective for micropropagation of various ornamental plants [12, 13, 28].

Other studies, shows that often caulogenesis and rhizogenesis do not both occur simultaneous onto the same culture media [1]. When an inocula, in which few cells are able respond to an inductive medium, is exposed subsequently to another media, the growth regulator composition of the first medium can have effects carried over to the second media [6, 18].

In an *in vitro* culture, hormonal balance and the combination of growth regulators can be fully controlled, - in this case 3-Indolebutyric acid mixed with thidiazuron - influence organogenesis within certain limits, by changing the concentration or ratio of them [8, 22, 29]. In order to select an efficient hormonal combination for micropropagation of the ornamental species *Begonia semperflorens* cv.

‘Ambassador’ White, both in an mineral basic medium culture MB - MS without growth hormone composition or in a mixed combination of IBA and TDZ at a concentration of 1.5 mg / l was investigated.

MATERIALS AND METHODS

In the present study, we aimed to develop an efficient *micropropagation* system, starting from regenerated small caulinar propagules with a diameter larger than 2 mm of *Begonia semperflorens* cv. ‘Ambassador’ White, collected as minicuttings.

The initial media used was based on standard Murashige-Skoog medium [10] because is the most commonly used media for the *in vitro* culture of *Begonia* [25], with an addition of PP (nicotinic acid) B₁ (thiamine) and B₆ (pyridoxine) vitamins in a concentration of 1 mg / l and growth regulators, 3-Indolebutyric acid (IBA) and thidiazuron (TDZ) at a concentration of 1.5 mg / l (each), single or in combination, as shown in Table 1

The mineral basic medium culture Murashige and Skoog was supplemented with 100 mg/l myo-inositol, 30 mg/l sucrose and 7 g/l Difco-Bacto agar. The culture medium was sterilized by autoclaving at 121°C for 20 minutes and the pH was adjusted to 5.8.

The nutrient media was poured in glass vials with a height of 8 cm and a diameter of 3 cm. The explants were inoculated in aseptic room and vials were sealed with sterile polyethylene folia and immobilized with rubber. In each variant 30 explants were cultured, 1 explant per glass recipient, after that were exposed at white fluorescent light, on the shelves at a luminous intensity of 16 μmol m⁻² s⁻¹ and a photoperiod of 16 hours light / 8 hours dark. The temperature varied at 23 ± 1°C.

All data were processed mathematically and compare to the control group (reference group) whose values were considered 100%, making way for the analysis of the average, standard deviation and variance using EXCEL functions, where the value of $p < 0.05$ were considered as significant difference.

Table 1. Growth regulators content of media used for *in vitro* regeneration of *Begonia semperflorens*.

Media	Growth regulators	Concentration (mg/l)
V ₀	Control group	Without growth regulators
V ₁	3-Indolebutyric acid	1.5 mg/l
V ₂	Thidiazuron	1.5 mg/l
V ₃	3-Indolebutyric acid + Thidiazuron	1.5 mg/l + 1.5 mg/l

RESULTS

Morphogenic responses of *Begonia semperflorens* cv. 'Ambassador' White vitroculture were observed at

90 days of culture and parameters considered were: diameter of multiple shoots clusters, number of leaflets, diameter of the largest leaflet, the number of buds per explant and diameter of largest *callus*.

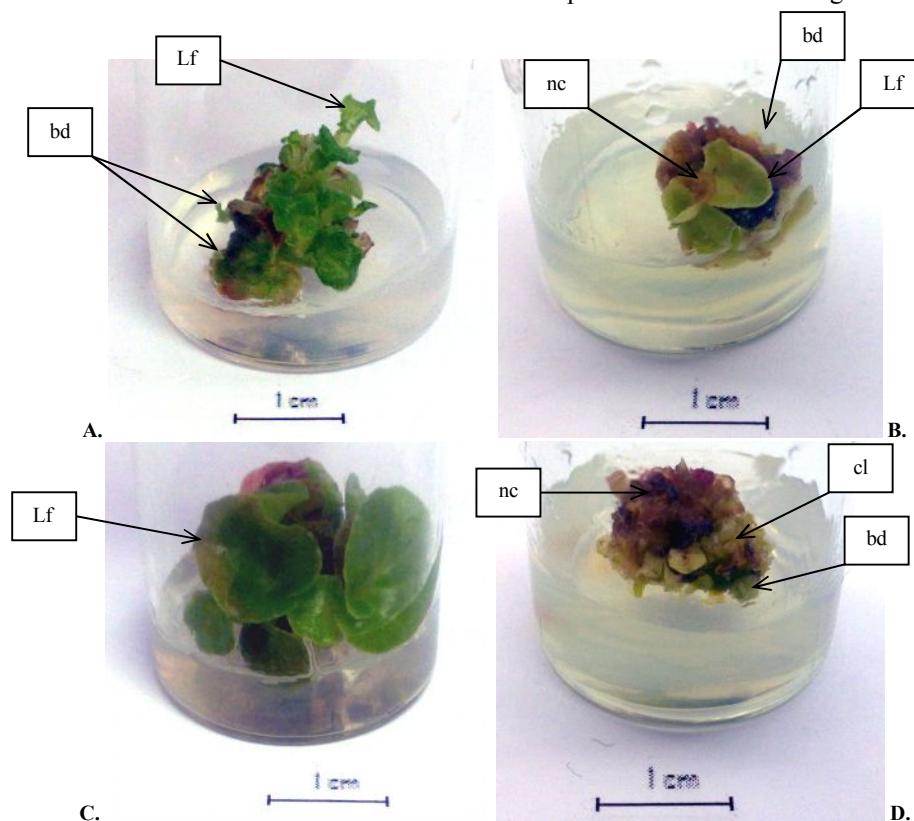


Figure 1. Image of *Begonia semperflorens* cv. "Ambassador" White inoculas at 90 days after their inoculation *in vitro*, on variants: V₀ - (control group) mineral basic medium culture MB - MS without growth regulators (A); V₁-V₃ - mineral basic medium culture MB - MS supplemented with growth regulators (B-D), where: Lf - new leaf; bd - bud; cl - callus; nc - necrosis.

The highest rate of formation of a rich mass *leaflet* was produced on variant V₂ (mineral basic medium culture MB - MS supplemented with the addition of 1.5 mg / l TDZ) (Table 2), which gave the best results on the induction of multiple well developed leaves.

Cytokines requirements are different depending on the species. Increasing cytokines concentrations of culture media in order to stimulate axillary shoots may lead to multiple shoots yield which can continue after subculturing on media without cytokines for elongation or rooting [12, 23], respectively in the induction media (for *calusogenesis*) [9].

After 90 days, of culture the *diameter of shoot clusters* exceeded the control group (over 7 mm), only onto variant V₂ (basic mineral medium culture MB - MS supplemented with the addition of 1.5 mg / l TDZ) with shoot clusters up to 13.3 mm with a distinct significant difference (Table 2) from statistical point of view. On the experimental variant V₃ (basic mineral medium culture MB - MS supplemented with a mix of

1.5 mg / l IBA plus 1.5 mg / l TDZ) the clones developed well-formed shoot clusters up to 11 mm, marking a decrease of 3%.

There were also differences between the *number of leaflets* (Fig. 3A), most recorded values were present to control V₀ (mineral basic medium culture MB - MS without growth regulators) on which well-distinguished countable 12.5 leaflets / explant were developed Variant V₂ (basic mineral medium culture MB - MS supplemented with addition of 1.5 mg / l TDZ), marked an increase of 25%, compared to the variant V₁ (basic mineral medium culture MB - MS supplemented with the addition of 1.5 mg / l IBA) formed 9.6 leaflets /explant, recording a deficit of 3.3% observing the presence of necrosis on the older leaves edges, respectively on variant V₃ (basic mineral medium culture MB - MS supplemented with a mix of 1.5 mg / l IBA plus 1.5 mg / l TDZ) were noticed less then 7 leaflet / explant, which represents a deficit of 30% presenting a significant difference (Table 2). These

results are in concordance with other authors publications [21], which reported that for the *in vitro* differentiation in some species of *Begonia*, it is absolutely necessary to supplement the culture medium with cytokines like in our case TDZ was used.

Some shoots from these clones remained small and the diameter of the largest leaflet on V₁ variant was 4.8 mm, with a deficit of 52% representing a distinct significant difference (Table 2), on variant V₂ were formed leaves with a diameter over 6.3 mm and on variant V₃ this parameter reached a maximum size of 6 mm. Their growing capacity and caulogenesis were influenced by each variant type of medium used in the micropropagation of this ornamental species.

The average number of buds onto V₀ variant was 4 buds/ explant and onto V₁ variant was 1.33 buds/

explant, meaning an decrease of 87.7% due to the presence of auxine; in the presence of cytokine (simple or mixed combination) onto media V₂ resulted 6.25 buds / explant, respectively onto media V₃ with 7.33 buds /explant, representing a deficit of 26.7% with a significant difference from statistical point of view (Table 2). The stimulative effects of Thidiazuron upon the shoot bud yeld was higher than those of usual cytokines the highest shoot bud formation being obtained on media supplemented with TDZ [17].

From all experimental variants studied, only onto the variant V₃ a callus mass with a diameter of 9 mm was produced. For callus formation may be due to the action of accumulated auxins which stimulates cell proliferation, especially in the presence of cytokines [23].

Table 2. The statistical processing of biometrical data of *Begonia semperflorens* cv. ‘Ambassador’ White inoculas, at 90 days after *in vitro* inoculation on MS medium culture (1962) without hormones and with different growth regulators, where 30 explants per variant.

Biometrics Statistics evaluation	Diameter of shoot clusters	Number of leaflets	Diameter of the largest leaflet	Number of buds	Diameter of the largest callus
Variant V₀					
$\bar{x} \pm S_{\bar{x}}$	0.72 ± 0.11	11.67 ± 4.13	0.67 ± 0.16	4.00 ± 1.41	-
s	0.1169	4.1312	0.1633	1.4142	-
S%	16%	35%	24%	35%	-
Variant V₁					
$\bar{x} \pm S_{\bar{x}}$	0.80 ± 0.10	9.67 ± 1.15	0.48 ± 0.09	1.33 ± 0.57	-
s	0.1000	1.1547	0.0957	0.5774	-
S%	12%	11%	19%	43%	-
±d	0.08	-2.00	-0.19	-2.67	-
p	ns	ns	**	**	-
Variant V₂					
$\bar{x} \pm S_{\bar{x}}$	1.33 ± 0.33	12.50 ± 11.12	0.63 ± 0.12	6.25 ± 2.98	-
S	0.3304	11.1206	0.1258	2.9861	-
S%	24%	88%	19%	47%	-
±d	0.61	0.83	-0.04	2.25	-
p	**	ns	ns	ns	-
Variant V₃					
$\bar{x} \pm S_{\bar{x}}$	0.97 ± 0.11	7.00 ± 2.00	0.60 ± 0.10	7.33 ± 2.08	0.09 ± 0.00
S	0.1155	2.0000	0.1000	2.0817	-
S%	11%	28%	16%	28%	-
±d	0.25	-4.67	-0.07	3.33	-
p	**	*	ns	*	-

Note: p (significance level of difference), ns (no significant difference), * - significant difference, ** - distinct significant difference, *** - very significant difference, $\bar{x} \pm S_{\bar{x}}$ (average (cm) ± standard deviation), S (difference deviation), S% (variability value), ±d (difference compared to the control – in cm).

DISCUSSIONS

There are studies which present some researches made on *Begonia* sp., using different parts of plant material [14, 26], where the period of culture *in vitro* also influence the development of various floral cultures [7, 10, 11, 24, 26].

In the case of this experiment we investigated the influence of IBA and TDZ, upon the regenerative capability of *Begonia semperflorens* cv. ‘Ambassador’ White explants after 90 days of *in vitro* culture. Our study reveals that the regenerative capability was different on each type of culture media used in this experiment and also that TDZ can stimulate induction

and multiplication of shoots either alone or in combination with other growth regulators [10].

The results achieved in this experiment, shows that the presence of growth regulators was stimulative for the morphogenetic expression of *Begonia semperflorens* cv. ‘Ambassador’ White inoculas, resulting in regeneration of both shoots and calluses. It is noted that biometric parameter values increase directly proportional with the addition of TDZ in the culture media.

Upon completion of the experiment the survival percentage of the regenerants in the culture medium without growth regulators (V₀) and the other variants of the culture under study, was between 50-70% and it was noted the appearance of necrosis.

Optimal medium culture proved to be the basic mineral medium culture MB - MS supplemented with 1 mg / l TDZ (V₂), on which were obtained with 38.6% more leaflets - compared with the control group - also the average length of newly formed shoot clusters exceeded in height with 4% in group similar bodies compared to control group V₀.

In conclusion, our results suggest that the *in vitro* propagation of *Begonia semperflorens* cv. 'Ambassador' White, using additions of cytokine like TDZ, (which stimulate caulogenesis), could be open a new way for an efficient micropropagation protocol for *Begonia* species with a better regenerative capability and plant yield.

Acknowledgements. This work carried out with the support of Plant Biotechnology Laboratory of the Department of Biology, Faculty of Sciences, University of Oradea.

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Received: 5 April 2011

Accepted: 19 May 2011

Published Online: 22 May 2011

Analele Universității din Oradea – Fascicula Biologie

<http://www.bioresearch.ro/revistaen.html>

Print-ISSN: 1224-5119

e-ISSN: 1844-7589

CD-ISSN: 1842-6433