INITIATION OF *Begonia erythrophylla* L. VITROCULTURE FROM AXILLARY BUDS

Julieta - Emilia ROMOCEA[*], Liviu POP[*], István GERGELY[**]

[*] *University of Oradea, Faculty of Science, Department of Biology, Oradea, Romania*
[**] *National Administration "Romanian Waters", "Crisuri" Water Basin Administration, Oradea, Romania.*
Corresponding author: Julieta - Emilia Romocea, University of Oradea, Faculty of Science, Department of Biology, 1 Universităţii Str., 410087 Oradea, Romania, phone: 0040259408448, e-mail: jromocea@uoradea.ro

Abstract. In our aim was to establish a short-term *in vitro* culture of *Begonia erythrophylla* L., different culture media compositions were tested: complex variants added with growth regulators and simplified modified media with Heller microelements and MS macroelements. Adventitious shoots elongating over 13 mm in length were efficiently obtained from axillary bud segments of the strain of begonia *Begonia erythrophylla* L. on MS basic mineral medium culture containing different concentration of growth regulators, in order to identify optimal culture conditions, which would facilitate the achievement of a vitroculture, allowing the *in vitro* culture of this studied species. On complex regeneration variant V_1 – mineral basic medium culture MB - MS supplemented with 1 mg/l BA, the rooting process was absent, but according to this carried out research, plantlets were obtained by rooting the elongated shoots on Murashige-Skoog (1962) basic mineral media containing 1 mg/l IBA, respectively a basic mineral medium culture without growth regulators, producing a much better organogenesis, where the phenomenon was greater in rooting process.

Keywords: *Begonia erythrophylla* L., cytokine, auxine, micropropagation

INTRODUCTION

Begonia genus (*Begoniaceae* Fam.) holds approximately 2000 varieties, originating in Brazil but in Europe it appeared in 1828, as ornamental plants [21]. Most of these varieties are herbaceous plants, lianas or rarely shrub form plants. *Begonia erythrophylla* L. is a native plant to humid tropical and wetland's as the South-East Asia, South America and parts of India [19]. Of the many species of the genus *Begonia*, over 200 species have a high commercial value, where: *B. semperflorens, B. rex, B. tuberhybrida, B. socotrana* etc. [23]. Begonias can be multiplied by seeds or vegetative naturally by rhizomes or tubers. Also, in the modern times, it can be multiplied artificially by minicuttings *in vitro* micropropagation, an effective and practical method [3, 4, 5]. Recent modern techniques of propagation, which could help growers to meet the demand of the horticultural industry in the next century, have been developed. *In vitro* regeneration of four *Begonia* genotypes, *B. semperflorens, B. rex, B. elatior* and on a hybrid of *Begonia* with unknown parents, 'Tiger', was carried out starting from leaf and petiole segments as explants, the BA containing MS basic mineral medium [15], quantitative differences among explants and genotypes were observed [7, 18]. Thin cell layer culture was applied for begonia micropropagation, MS basic mineral medium (Murashige - Skoog, 1962) supplemented with 0.2 mg/l NAA and 0.2 mg/l BA was demonstrated to be optimal for shoot regeneration from tTCLs [12, 13, 17, 20]. It has been studied the rooting and caulogenesis phenomenon on explants grown from fragments of *Begonia erythrophylla* L. stems, to make the link between developmental stages and primordial explant physiological requirements [1]. Caulogenesis and rhizogenesis were studied in cultured petiole explants of *Begonia erythrophylla* in order to link the developmental stages of primordia initiation with the physiological requirements of the explant. Also, the influence of light quality on organogenesis *in vitro* was investigated using *Begonia erythrophylla* petiole explants [2]. Different concentrations of BA with NAA were investigated to optimize regeneration of *Begonia elatior* cv. Toran orange [14]. In the case of *Begonia rex* L., the regenerative cells are placed under the skin, being located in the glandular trichome, these cells will form a meristem, which will generate, then, seedlings, as in the case of *Saintpaulia*. The commercial micropropagation efficiency programs for *Begonia* sp. minicuttings depends on the production of adventitious buds, which facilitates a more efficient rooting and acclimatization of plantlets regenerated from vitrocultivated explants [8]. The regeneration of adventitious shoots from petiole segments of *Begonia* sp., is also possible on a solid culture medium Murashige – Skoog supplemented with hormonal addition of 0.1 mg/l NAA and 0.5 mg/l BA. With a low concentration of 0.1 mg/l BA, less strains were regenerated from explants, but increasingly more stem in exchange on a culture medium with the addition of 0.1 mg/l BA were produced 50% more buds, which were regenerated in explants larger than 1 cm from the culture medium with a higher concentration of BA (0.5 mg/l) [22]. The inducing and development of adventitious caulogenic and rhisogenetic phenomena are aspects that can be materialized by the use of adequate combinations between cytokine and auxine, and is also a known fact that different types of explants contribute to the achievement of the *in vitro* culture with an endogenous content in phytohormones [10]. In the *in vitro* micropropagation process, it was achieved on plants the usage of exogene phytohormones, constituting a decisive element for their multiplication feasibility [6]. It must be noticed also the fact that rhizogenesis in *Begonia* sp. was achieved in the absence of an exogenous auxinic source, too.

In the present study, we aimed to develop an efficient micropropagation system for *Begonia erythrophylla* L., using axillary buds from the mother plant, inoculated *in vitro* on MS basic mineral medium (Murashige - Skoog, 1962) with added growth regulators to bring - at their level - the formation of roots and stems.

MATERIALS AND METHODS

To mount this experiment we used as plant material, used for the collection of explants, axillary buds, which consisted in fragments with size of 0.6 mm - 1 cm, taken from mother plants of *Begonia erythrophylla* L. grown in pots, in greenhouse conditions. The period of axillary buds harvesting was before the summer season (in March). *Begonia erythrophylla* L. minicuttings sizing was realized according to the scheme from Fig. 1. The fragments shown in Fig. 1C were sterilized by the following procedure: minicuttings were kept under running tap water around 2-3 hours, after which they were immersed in 96° alcohol for 30 seconds, stirring continuously, then they were washed with sterile water, followed by an operation of dipping them in sodium hypochlorite solution 0.5% plus 3-4 drops of Tween 20, mixed - 1:1 - with sterile water, stirring it continuously, were maintained for 15 minutes, and then it was washed with sterile water every five minutes, five times. The plant material was deposited in Petri dishes on filter paper, both previously sterilized in the oven at 170° C for 2 hours. In a laminar flow hood perimeter with sterile air, put into operation, all the affected and bleached portions after sterilization were removed. The plant material was inoculated on the medium culture, number of used 15 explants per variant, according to described experimental variations listed below.

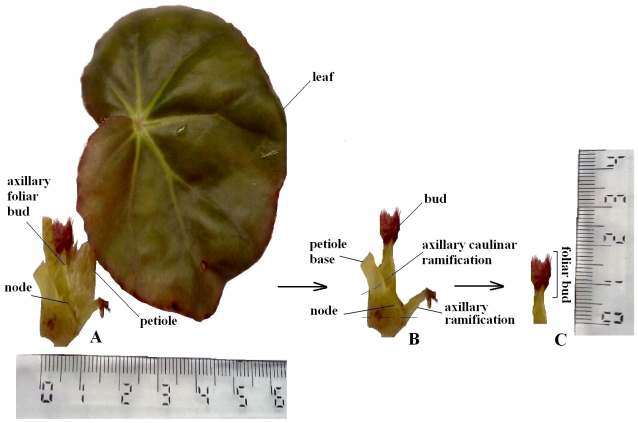

Figure 1. Sectioning scheme of *Begonia erythrophylla* L. minicuttings; **A** and **B** – axillary bud; **C** – minicutting.

The substrate layer was composed from a modified mineral basic medium culture (BM) and Fe EDTA, macroelements Murashige-Skoog (MS) (1962), Heller micronutrients, vitamins that were added: pyridoxine HCl (1 mg/l), thiamine HCl (1 mg/l) and nicotinic acid (1 mg/l), 100 mg / l myo-inositol, 30 g/l sucrose and 7 g/l agar-agar, the medium pH was adjusted to a value of 5.8. To this mineral basic medium culture, were added different growth regulators, representing the following variants: V_0 – (control group) – mineral basic medium culture MB - MS without growth regulators; V_1 – mineral basic medium culture MB - MS supplemented with 1 mg/l 3-Indolebutyric acid (IBA); V_2 – mineral basic medium culture MB - MS supplemented with 1 mg/l N^6-benzyladenine (BA); V_3 – mineral basic medium culture MB - MS supplemented with a mix of 1 mg/l N^6-benzyladenine (BA) plus 1 mg/l 3-Indolebutyric acid (IBA). The final medium culture, it was distributed - with a sterile distributor - in sterile glass vials, which were autoclaved at a pressure of 1 atmosphere at 120°C for 20-25 minutes. In the cooled vials with medium culture, the minicuttings were inoculated, one by one, and then the vials were obturated with transparent folia, immobilized with elastic at the bottles mouth, they were transferred into the growth chamber and placed on shelves, maintained at a temperature that varied between 22-25ºC with 16 hours light / 24 h (1500 lux light intensity) emitted light by white fluorescent tubes.

After *30 days* biometric measurements were performed, which consisted in: *number* and *average length of stems, number* and *dimension of leaves, number* and *average length of roots*, respectively regenerated organs assessments of the vitroplant consisted from minicuttings.

All data were processed mathematically, data reported to the control group (reference group) whose values were considered as 100%, one way analysis of average, standard deviation and variance, Excel Spreadsheet Program functions were used. P value <0.05 were considered as very significant.

RESULTS

The *Begonia erythrophylla* L., at *30 days* after initiation of culture, formed a large number of leaves, respectively roots, except only variant V_2 – mineral basic medium culture MB - MS supplemented with 1 mg/l BA, where there has been not formed any roots (Fig. 2). *Number of stems* presented values much lower because the witness has been developed most vitroplantlets. Only on variants V_1 – mineral basic medium culture MB - MS supplemented with 1 mg/l IBA regenerated 2 stems / inoculum registering a value of 73.33% presenting insignificant values (Table 1), respectively on variant V_3 – mineral basic medium culture MB - MS supplemented with a mixing of 1 mg/l BA plus 1 mg/l IBA a value of 66.66%.

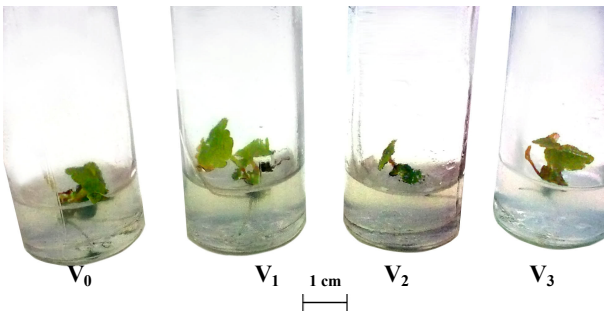

Figure 2. Image of *Begonia erythrophylla* L. vitroplantlets at *30 days* after their inoculation *in vitro*, on variants: V_0 - (control group) mineral basic medium culture MB - MS without growth regulators; V_1 - mineral basic medium culture MB - MS supplemented with 1 mg / l 3-Indolebutyric acid (IBA); V_2 - mineral basic medium culture MB - MS supplemented with 1 mg / l N^6-benzyladenine (BA); V_3 - mineral basic medium culture MB - MS supplemented with a mix of 1 mg / l N^6-benzyladenine (BA) plus 1 mg / l 3-Indolebutyric acid (IBA).

The *average length of stems* of vitroplantlets varied between 1.1-1.4 cm with a difference of -0.25 cm compared to the control on variant V_1 – mineral basic medium culture MB - MS supplemented with 1 mg/l IBA, respectively on variant V_3 – mineral basic medium culture MB - MS supplemented with a mixing of 1 mg/l BA plus 1 mg/l IBA, where the vitroplantlets regenerated stems with a length between 1.1-1.3 cm with a difference of -0.42 cm and presenting a very significant difference, but on variant V_2 – mineral basic medium culture MB - MS supplemented with 1 mg/l BA, the *average length of stems* it was 1 cm recording a distinct significant difference from statistical point of view (Table 1), longest were observed on control group, where they reached a maximum size of 1.2-1.4 cm.

Tabel 1. The statistical processing of biometrical data of *Begonia erythrophylla* L. vitroplantlets, at *30 days* after *in vitro* inoculation on a modified MS medium culture (1962) without hormones and with different growth regulators, where: the inocula of variants V_0 - (control group) mineral basic medium culture MB - MS without growth regulators; V_1 - mineral basic medium culture MB - MS supplemented with 1 mg / l 3-Indolebutyric acid (IBA); V_2 - mineral basic medium culture MB - MS supplemented with 1 mg / l N^6-benzyladenine (BA); V_3 - mineral basic medium culture MB - MS supplemented with a mix of 1 mg / l N^6-benzyladenine (BA) plus 1 mg / l 3-Indolebutyric acid (IBA).

Biometrics / Statistics evaluation	Stems number	Stems length	Leaves number	Leaves dimension	Roots number	Roots length
Type V_0						
$\bar{x} \pm S_{\bar{x}}$	0.80 ± 0.41	1.22 ± 0.09	1.4 ± 0.50	0.16 ± 0.08	1.53 ± 0.51	1.2 ± 0.16
S	0.4140	0.0981	0.5074	0.0814	0.5164	0.1634
S%	3.45%	0.81%	4.22%	0.67%	4.55%	1.29%
Type V_1						
$\bar{x} \pm S_{\bar{x}}$	0.73 ± 0.45	0.97 ± 0.51	1.6 ± 1.05	0.78 ± 0.52	1.2 ± 1.01	0.95 ± 0.49
S	0.4577	0.5133	1.0556	0.5244	1.0142	0.4955
%	73.33%	91.25%	160%	78.12%	120%	88.75%
S%	4.16%	5.02%	9.59%	4.76%	9.21%	4.85%
d ±	-0.07	-0.25	0.2	0.62	-0.33	-0.25
p	ns	*	ns	**	ns	*
Type V_2						
$\bar{x} \pm S_{\bar{x}}$	0.4 ± 0.50	0.44 ± 0.48	0.53 ± 0.74	0.29 ± 0.35	-	-
S	0.5070	0.4881	0.7432	0.3549	-	-
%	40%	41.25%	53.33%	29.37%	-	-
S%	8.45%	8.07%	12.38%	5.91%	-	-
d ±	-0.40	-0.78	-0.87	0.14	-	-
p	**	***	***	***	***	***
Type V_3						
$\bar{x} \pm S_{\bar{x}}$	0.67 ± 0.48	0.80 ± 0.53	1.47 ± 1.12	0.77 ± 0.54	0.87 ± 0.74	0.50 ± 0.38
S	0.4879	0.5370	1.1255	0.5498	0.7432	0.3836
%	66.66%	80%	146.66%	76.87%	86.66%	46.87%
S%	4.87%	5.60%	11.25%	5.49%	7.43%	3.91%
d ±	-0.13	-0.42	0.07	0.61	-0.66	-0.70
p	ns	**	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 in cm ± standard deviation), S (standard deviation), % (average in %), S% (variability value), ±d (difference compared to the control – in cm).

In terms of the *number of leaves*, we noted that most leaves were present on control variant and on the variants V_1 registering an increase of 60%, respectively on variant V_3 – mineral basic medium culture MB - MS supplemented with a mixing of 1 mg/l BA plus 1 mg/l IBA with an increase of 46.66%, where the vitroplantlets generated 2-3 leaves/vitroplantlet, but very significant differences were obtained variant V_2 –

mineral basic medium culture MB - MS supplemented with 1 mg/l BA. *Dimension of leaves* registered the most increases on variant V_1 – mineral basic medium culture MB - MS supplemented with 1 mg/l IBA with a distinct significant difference, respectively on variant V_3 – mineral basic medium culture MB - MS supplemented with a mixing of 1 mg/l BA plus 1 mg/l IBA registering a value of 76.87%, very significant differences were noted on variant V_2 – mineral basic medium culture MB - MS supplemented with 1 mg/l BA. The longest roots we obtained on variant V_0 where the *Begonia erythrophylla* L. vitroplantlets roots did reached a maxim of 1.4 cm, followed by variant V_1 – mineral basic medium culture MB - MS supplemented with 1 mg/l IBA registering a value of 88.75% - the *length of roots* - was 1.3 cm with a significant difference of -0.25 cm and on variant V_3 – mineral basic medium culture MB - MS supplemented with a mixing of 1 mg/l BA plus 1 mg/l IBA presenting a very significant difference.

DISCUSSIONS

There are studies which present some researches made on *Begonia* sp., somaclonal variation is wide spread among tissue culture-derived regenerants [9, 11], plant regeneration via organogenesis or protoplasts often leads to more somaclonal variations; the length of culture period also influences somaclonal variations [11].

Also it was investigated the influence of two cytokines on somaclonal variations in the ornamental plants of *Begonia elatior* and *Saintpaulia ionantha* L. [16], which were selected carefully for uniformity in morphology, using excised leave disks and cultured according to [9] on MS medium supplemented with different concentrations of cytokines and auxins. The cytokinin influence on somaclonal variation was observed by selecting BA for *Begonia* (like in our case) and kinetin and zeatin for *Saintpaulia.* A total of 200 plantlets of *Begonia* on each MS-I and MS-II, and 500 plantlets of *Saintpaulia,* were regenerated on each medium MS-II and MS-III, all possible care was taken to select uniform control plants so that somaclonal variation could be studied in the regenerated plants of both the species.

In the case of this experiment to initiate an *in vitro* culture of *Begonia erythrophylla* L., from our study it has been revealed that the initiation of this ornamental plant is possible. Also, it was followed by the *Begonia erythrophylla* L. vitroculture, the inoculations being made on four types of culture media, which were studied for *30 days* of initiation at the level of respective vitrocultures, most of them being on control variant, have shown a good regenerative capacity, on the variant V_3 (mineral basic medium culture MB - MS supplemented with a mix of 1 mg/l BA plus 1 mg/l IBA), V_1 (mineral basic medium culture MB - MS supplemented with 1 mg/l IBA) remarking regeneration of stems, leaves and roots, respectively on variant V_2 where the vitroplantlets stems were regenerated very weakly, and the rhizogenesis phenomenon wasn't present. According to this carried out research, we proved that *Begonia erythrophylla* L. is a species easily initiated *in vitro* from axillary bud fragments, used as explant, the initiation accomplished with success, so we finally reached the conclusion that the recommended culture medium, generally used, is the Murashige-Skoog (1962) V_0 (medium without growth regulators). Also, the growth regulators used in this experiment are compounds belonging to different categories of organic substances, or are synthetic plant hormones (e.g. IBA, BA), or are compounds that mimic the effect of plant hormones, but in this experiment showed that it can regenerate the same way. Thus, for this experiment, we observed that the ability to shoot the axillary bud could be realized also on a medium culture without growth regulators, depending on the explant type, depending on the composition of culture medium with growth regulators (such cytokine - BA or an auxine – IBA, in our case), from this buds, future plantlets were regenerated.

In conclusion, our results suggest the micropropagation of the selected species of ornamental plant - *Begonia erythrophylla* L., using additions of cytokines have an important role for rhizogenesis, but it is difficult to predict the number of micropropagated cycles or generations when the genetic stability of this trait may be lost. In the same time, we will try to study more possibilities in this species of *Begonia erythrophylla* L. or even other *Begonia* sp. to establish sterile cultures with a better regenerative capacity, which is a significant step in the *in vitro* development programme for the conservation of rare and endangered begonias.

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