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INITIATION OF *BEGONIA SEMPERFLORENS* CV. ‚AMBASSADOR' WHITE VITROCULTURE FROM PLANT APEX OF zygote ORIGIN

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ABSTRACT.
The initiation of *Begonia semperflorens* cv. 'Ambassador' White vitroculture was made by *in vitro* cultivation of some explants consisting in apical minicuttings, approx. 3-4 mm size, taken from *30 days* old seedlings, derived from germinated seeds in aseptic Petri capsules, on filter paper moistened with sterile water. Explants were inoculated and grown – for *30 days* – on Murashige-Skoog mineral medium (1962), modified, with added vitamins, sucrose, agar-agar, with or without growth regulators, namely the addition of *indolebutyric acid* (IBA) and/ or with thidiazuron (TDZ) 0.5 mg/l each. The minicuttings consisted of upper side of *hypocotile*, which held the bud strain of plant, the two epigeal cotyledons being inserted into its basal zone.
After *30 days in vitro* culture, the best results in terms of *phylogenesis* were obtained from the mineral basic medium culture with the addition of 0.5 mg / l TDZ, the number of regenerated leaves was much higher than that recorded on the other experimental variants. But the most balanced regeneration on buds, leaves respectively, as well as on roots was obtained from mineral basal medium culture supplemented with 0.5 mg / l TDZ in combination with 0.5 mg / AIB. If the culture was present 2.5mg /l *2.4*-dichlorophenoxyacetic *acid* (*2,4-D*), the morphogenesis consisted only in *calusogenesis*.
Keywords: *Begonia semperflorens* cv. 'Ambassador' White, cytokine, auxine, micropropagation.

INTRODUCTION

Using *in vitro* culture techniques on plants has become a routine procedure for mass propagation of many plant species, especially orchids, ferns, ornamental trees, fruit trees, but strawberries, potatoes, etc. (Loo, 1982; Murashige, 1974).

Most studies on micro propagation of begonias by tissue culture were made on solidified medium culture. But Takayama and Misawa (1981, 1982), and Simmonds and Werry (1987) have succeeded - in *Begonia x hiemalis* – the regeneration of plantlets *in vitro* from bud type explants, under conditions of their cultivation in liquid medium.

In order to initiate *Begonia* vitroculture, explants from the mother plants were generally used - plants which were grown in septic, natural conditions – by using fragments of leaves, stems, flower components etc. For the neogenesis of the buds at their level, a supplementing of the medium of culture with a mixture of auxine and cytokine is benefic. (Cachiţă et al., 2004).

Takayama and Misawa (1981, 1982) found that by adding 1.3 μM *bensiladenine - (BA)* or 4.6 μM *kinetin - (KIN)* in combination with 5.4 μM *α naphtilacetic acid - (NAA)* to the culture medium the regeneration of many other vegetative buds or somatic embryo induction and regeneration of plantlets from them – at the level of bud type explants of *Begonia* – was generated. The development of adventitious, multiple buds of *Begonia x hiemalis* explants *in vitro* was greatly stimulated in the cultivation on liquid medium (Takayama and Misawa, 1981, 1982; Simmonds and Werry, 1987). This type of culture permitted the use of a large scale of containers for medium culture, equipped with mechanical agitators or air bubbles, suitable for micropropagation of plantlets at a large scale (Takayama and Misawa, 1981).

Similar results were obtained by Roest (1977), Mikkelson and Sink (1978), Bigot (1981), Reuter and Bhandari (1981), etc. In other articles published by Ringe (1972), Wellander (1977, 1981), and by Appelgren (1984) it is asserted that - on *Begonia x hiemalis* - there is the possibility of initiating vitrocultures from floral stem type explants, using lower concentrations of cytokine such as 0.22 μM BA or even only 0.08 to 0.88 μM BA.

Among the *Begonia* species that had been *in vitro* micropropagated it is worth mentioning: *B. cheimantha* (Heide, 1967), *B. evansiana, B. sutherlandii, B. teusheri x coccinea, B. socotrana* (Ringe and Nitsch, 1968), *B. rex* (Arora et al., 1970), *B. richmondensis* (Ringe, 1972), *B. franconis* (Berghoef and Bruinsma, 1980), *B. hiemalis* (Reuter and Bhandari, 1981), *B. tuberhybrida* (Takayama and Misawa, 1982; Peak and Cumming, 1984), *B. venosa* (Pierikos and Tettero, 1987), *B. erythrophylla* (Romocea et al., 2010) etc. It appears from this list that *B. semperflorens* is not among *Begonia* species that were used to make *vitrocultures*. Therefore, in our studies we sought to initiate a *Begonia semperflorens* cv. 'Ambassador' White *vitroculture*, and perform a series of experiments with this species, using apical minicuttings taken from plants derived from embryos zygotes, at *30 days,* obtained by aseptic seed germination. Minicuttings consisted in two cotyledons - inserted on the terminal hypocotyls - having joined related the apical bud.

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MATERIAL AND METHOD

To obtain plantlets of *Begonia semperflorens* cv. 'Ambassador' White we germinated pelleted seeds from Cripple Creek Creations Inc in aseptic conditions. For this, they were sterilized with 0.25% sodium hypochlorite solution, to which 2 drops of "Tween 20", 100 ml of disinfectant were added. In this solution the seeds were maintained for 2 minutes, being constantly agitated, after which the disinfectant was decanted and the seeds were washed in several baths, a 1 minute each, with sterile water, then they were placed in sterile Petri capsules on aseptic filter paper moistened with distilled, sterile water. For *30 days*, germination took place in the growth chamber, under 16 hours light /24 hours on fluorescent white light and at a temperature of 24 º C ± 2 º C. Every two days, in laminar flow hood perimeter of sterile air, in operation, the seeds – respectively the seedlings - were sprayed with distilled, sterile water. On the *30th day* from placing the seeds to germination, explants were taken from the seedlings, consisting of the hypocotyls apical area, having the two cotyledons attached (which are epigeal type), and the seedling bud being located on the top of hypocotyls (Fig. 1 B and C).

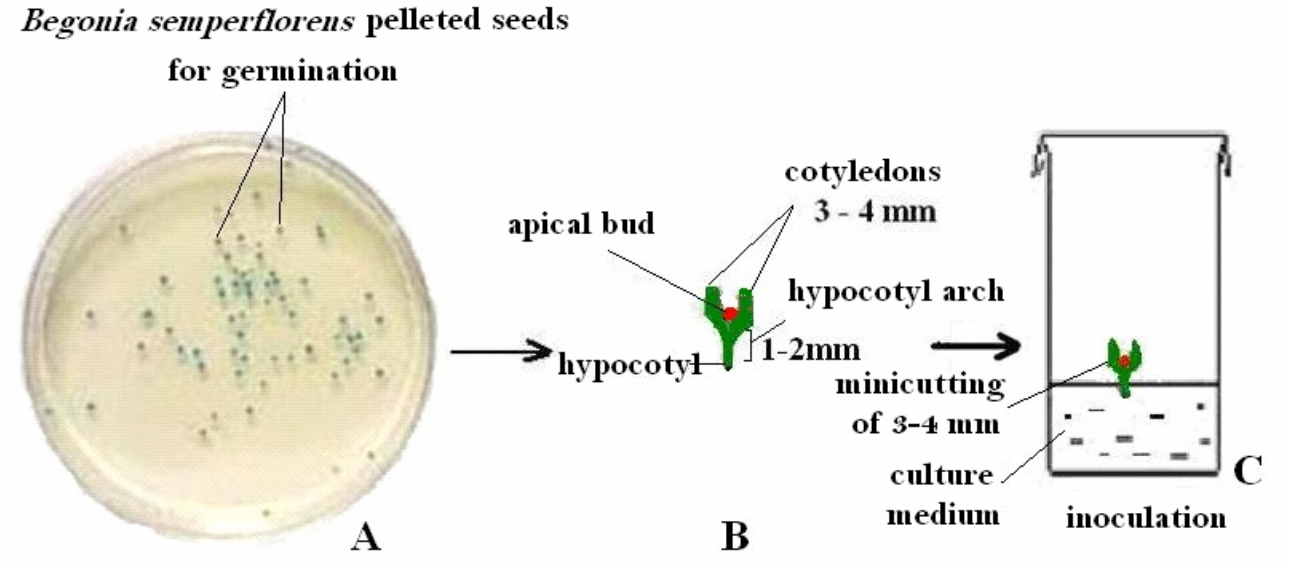


Fig. 1. Schematic representation of how to obtain explants of *Begonia semperflorens* cv. 'Ambassador' White, consisting of the hypocotyls apical area attached with two epigeal cotyledons, which have - between them – the apical bud, which constituted a minicutting, taken from seedlings of zygote origin, grown from germinated seeds under aseptic conditions (where: **A** - Petri dish with seeds germinated on filter paper; **B** - detail on the constitution of minicuttings apical type explants; **C** - inoculating explants – apical minicuttings - in aseptic medium cultures).

The culture medium on which the explants were inoculated and grown consisted of macro and micronutrients plus FeEDTA, basal mineral culture medium Murashige-Skoog (1962) (BM-MS), modified by us, with added vitamins: niacin, thiamine and pyridoxine 1 mg / l of each, 100 mg/ l myo-inositol, 30 g / l sucrose and 7 g / l Difco Bacto agar, with or without growth regulators (Table 1), pH was adjusted, previously by autoclaving, at the value of 5.8. After preparation, the culture media were distributed in glass recipients, 8 cm high and a diameter of 3 cm, which were obturated with cotton plugs. Their sterilization was made by autoclaving for 30minutes at a temperature of 121 ° C. After cooling the culture medium, inoculation was performed in an aseptic room, in the perimeter of laminar flow hood area of sterile air into service, using instruments to dry and flamed prior presterilised before each inoculation.

After inoculation, the obturation of the recipients was made with transparencies, colorless polyethylene, previously sterilized with 70 °alcohol, fixed with elastic. The incubation and growth of explants was performed by exposing the recipients to fluorescent white light, the recipients being placed on shelves, at a light intensity of 1700 lux and a photoperiod of 16-hour light / 8 hours dark. The temperature in the growth room ranged from 23 ° C and 27 ° C.

Tabel 1. The organization of the experimental variants according to growth regulators used as admixture to basal mineral culture media, Murashige-Skoog (1962).

Experimental variant code	Growth regulators	Concentration of growth regulators introduced in medium (mg/l)
V_0	Medium without growth regulators (Control group)	-
V_1	Thidiazuron (TDZ)	0.5 mg/l
V_2	Indolebutyric acid (IBA)	0.5 mg/l
V_3	Indolebutyric acid (IBA) + Thidiazuron (TDZ)	0.5 mg/l + 0.5 mg/l
V_4	2,4-dichlorophenoxyacetic acid (2,4-D)	2.5 mg/l

After *30 days* from the initiation of vitroculture from apical bud type, taken from the apex of plants from zygotes origin, we performed the biometric measurements of the explants regenerated vitrocultured explants. These measurements consisted in the determination of: the *average length and width of the largest leaf*, and *longest root*, and the *average number of roots and leaves* of the explants, in the case of *callus*, regenerated from explants grown on medium with 2,4-dichlorophenoxyacetic acid (2,4-D), we proceeded to measure their diameter.

The biometric results were statistically processed and the average values – on every trait - were reported at similar data recorded at the level of regenerated organs from explants grown on medium without growth regulators, V_0 control group, of which values were considered of reference, as 100%. The statistical calculation based on the average analysis and its standard deviation and variance was done using EXCEL functions, in the case in which the values of $p < 0.05$ were considered as being very significant differences. The results were plotted in percentage values.

RESULTS AND DISCUTIONs

On the *30th day* after the inoculation of *apical minicuttings* (approx. 3-4 mm) taken from plantlets of *Begonia semperflorens* cv. 'Ambassador' White (Fig. 2) zygote origin, on basal mineral medium culture Murashige-Skoog (1962) (BM-MS), both the control sample (V_0) – medium without growth regulators - and the variants V_1-V_3, the observations indicated morphogenesis at the level of fitoinocules found mainly at the *leaf neogenesis*, while the basal mineral medium culture Murashige-Skoog (1962) (BM-MS) with 2.5 mg / l 2,4-D from explants regenerated only green *callus*.

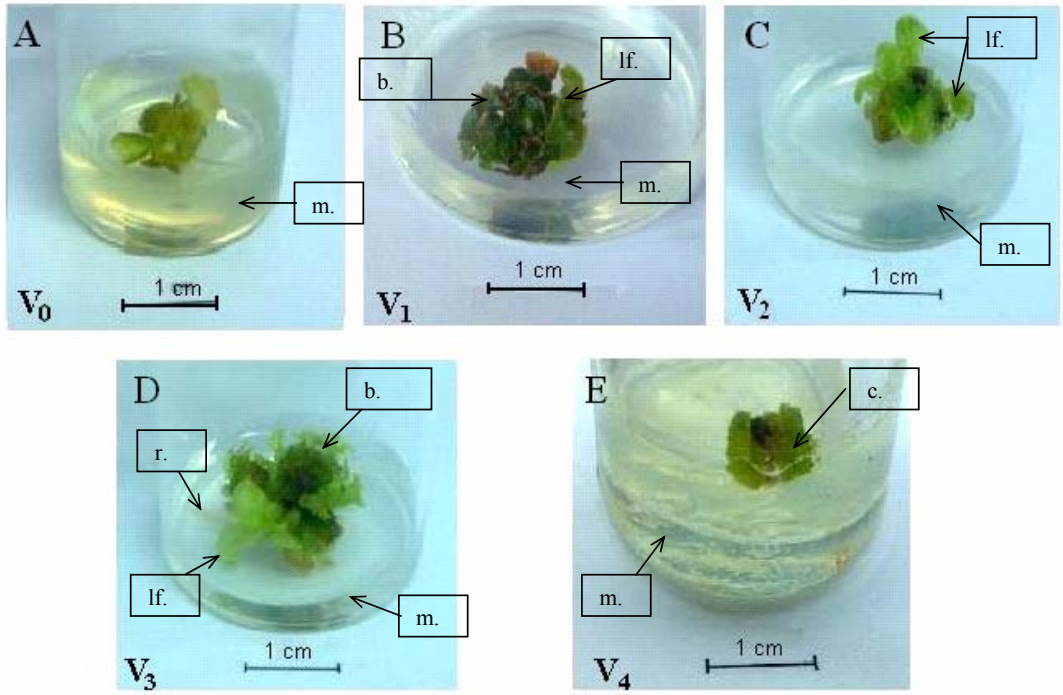


Fig. 2. Pictures of the *Begonia semperflorens* cv. 'Ambassador' White vitroculture initiated from apical minicuttings on the *30th day* of their *in vitro* inoculation on following culture media: V_0 - (control group) basal mineral medium culture (BM) - MS without growth regulators (A); V_1 - BM - MS supplemented with 0.5 mg / l thidiazuron (TDZ) (B); V_2 - BM - MS supplemented with 0.5 mg / l i*ndolebutyric acid* (IBA) (C) V3 - MB - MS supplemented with 0.5 mg / l tidiazuron (TDZ) in combination with 0.5 mg / l i*ndolebutyric acid* (IBA) (D) and V_4 - BM - MS supplemented with 2.5 mg / l *2,4*-dichlorophenoxyacetic *acid* (*2,4-D*) (E), (where: lf – leaf; c – callus; m - culture medium; b – buds; r - root).

On culture medium with the addition of 0.5 mg / l TDZ (V_1 variant) (Fig. 3A and B) (V_1), not the *length or width of the leaf* was great, but the *number of leaf* of explants that was regenerated that reached maximum values in relation to variants V_2 - V_4 the increase being of 250% . These new leaves resulted probably from the many buds that had been regenerated at the level of inocules from caulinar minicuttings, apical type.

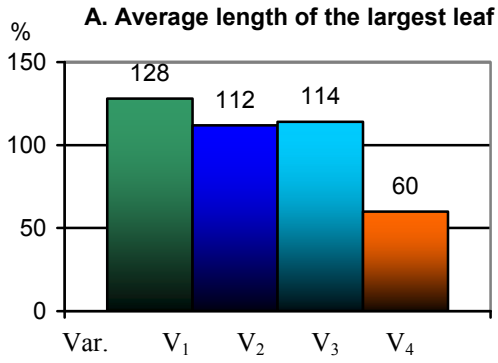
However, as shown in figure 3, biometric measurements showed that, especially on variant V_1- BM-MS supplemented with 0.5 mg / l TDZ, the *length of the largest leaf* showed an increase of 28% (Fig. 3A) in relation to their size in similar cultures of *Begonia semperflorens* cv. 'Ambassador' White regenerated and grown on medium culture without growth regulators V_0, control group, considered as reference values, respectively as 100%; in the case of variantV_3 - BM-MS supplemented with 0.5 mg / l TDZ mixed with 0.5 mg l / IBA (Fig. 3A) than the control group (V_0), the largest leaf waist presented an increase of 14% .

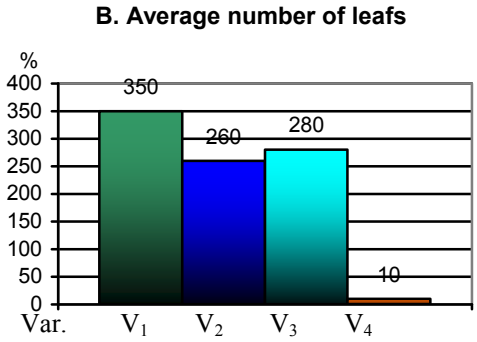
Also on variant V_1, the *average width of the largest leaf*, regenerated at the level of inoculum, the average value of 10.3 mm was achieved (data were highly statistically significant), showing an increase of 8% value of this parameter marked in the control group(V_0), however, the variant V_3 - BM - MS supplemented with 0.5 mg / l TDZ in combination with 0.5 mg / l IBA presented distinct significant difference, with less than 12 % to the size of this parameter marked the witness (V_0) was recorded in terms of *average width of the largest leaf*.

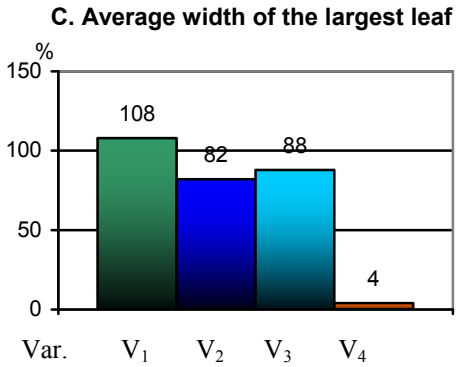
But in terms of *highest average of roots* (Fig.3D) regenerated on the level of each minicutting cultivated on culture medium V_2 - BM - MS supplemented with 0.5 mg / l IBA was 2-3 per explant, marking an increase of this parameter by 180% compared to control group (V_0) medium without growth regulators, distinct difference being statistically significant. The V_3 variant - BM - MS supplemented with 0.5 mg / l TDZ mixed with 0.5 mg l / IBA, were formed only 1-2 roots / explants at this parameter is 40% increase, compared to the control group. The variants that contained the substrate only 0.5 mg / l TDZ (V_1 variant) or 2.5 mg / l 2.4-D (V_4 variant) risogenesis was absent.

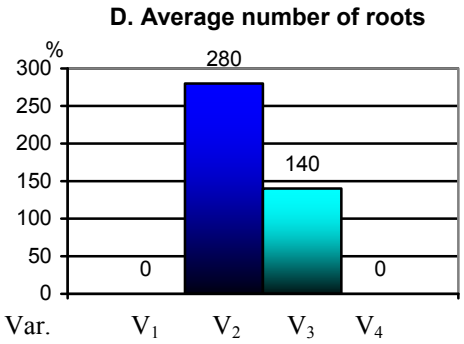
The *average length of the longest root* (Fig. 3E) was 11.6 mm, recorded on variant V_2 – BM - MS supplemented with 0.5 mg / l IBA, to witness an increase of 16% (the data were statistically distinct significant) and the variant V_3 - BM - MS supplemented with 0.5 mg / lTDZ mixed with 0.5 mg l / IBA, the *average length of the longest root* was 10.2 mm (data proved to be statistically significant) marking an increase of only 2% to the control group(V_0) (Fig. 3E).

Regeneration of *callus*, at the explants was observed only in apical minicuttings inoculated on medium variant V_4 - BM - MS supplemented with 2.5 mg / l 2.4-D, an *average diameter* of 7.2 mm.









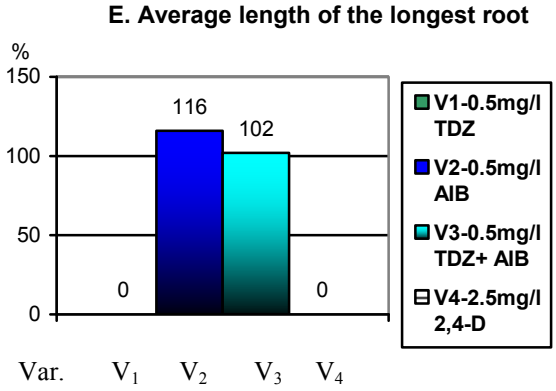


Fig. 3 Morphogenesis of the *Begonia semperflorens* cv. 'Ambassador' White vitroculture initiated from apical minicuttings taken from seedlings derived from germinated seeds in aseptic conditions (for *30 days)* performed on Murashige-Skoog basal mineral medium culture (1962) (BM - MS) without growth regulators (V_0), or BM - MS supplemented with 0.5 mg / l thidiazuron (TDZ) (V_1 variant) MB - MS supplemented with 0.5 mg / l indolebutyric acid (IBA) (V_2 variant); BM - MS supplemented 0.5 mg / l thidiazuron (TDZ) with a mixture of 0.5 mg / l indolebutyric acid (IBA) (V_3 variant); BM - MS supplemented with 2.5 mg / l 2,4-dichlorophenoxyacetic acid (2,4-D) (V_4 variant), plotted data are expressed in percentage values obtained by reporting the average parameter values similar data from biomeasured vitroplantlets to control group (V_0) - group without growth regulators - considered as reference values, respectively 100 %.

The fact that neither the *length nor width of the leafs* was not much increased, compared with similar explants regeneration on medium without growth regulators (V_0), prove that TDZ's presence in the medium culture led to a considerable stimulation of *phylogenesis* process respectively a neogenesis of buds as leaf generators.

CONCLUSIONS

1. Caulinar minicuttings, apical type taken from seedlings of *Begonia semperflorens* cv. 'Ambassador' White, the embryos derived from germinated seeds under aseptical condition, on filter paper moistened with distilled, sterile water, harvested in the *30th day* of germination, well suited for initiating a *in vitro* culture, both in terms of their inoculation on basal mineral Murashige-Skoog (1962) medium culture, modified by us, with added vitamins: niacin, thiamine and pyridoxine, 1 mg / l of each, 100 mg / l myo-inositol, 30 g / l sucrose and 7 g / l Difco Bacto agar, without growth regulators (control group), and especially the similar medium culture, but with thidiazuron (TDZ) at concentrations of 0.5 mg / l.
2. The presence in the culture medium of 0.5 mg / l TDZ stimulated phylogenesis with 350% for leaf neogenesis registered at minicuttings grown on culture medium without growth regulators, considered the reference values as 100%. I*ndolebutyric acid* (IBA) mixture - in concentration of 0.5 mg / l with thidiazuron (TDZ) 0.5 mg / l - stimulated both phylogenesis, marking an increase of 280%, the *number of leaf* regenerated at explants, also *risogenesis* and with an increase of 140% compared to those registered for the control parameter values.
3. Administration only i*ndolebutyric acid* (IBA) as the unique growth regulator present in the culture medium, stimulated both risogenesis and regeneration of roots and its length.

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