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REACTION COMPARISON OF BEGONIA SEMPERFLORENS cv. 'AMBASSADOR' WHITE PHYTOINOCULA MINIPROPAGULE TYPE IN THE PRESENCE OF THIDIAZURON AND / OR INDOLEBUTYRIC ACID

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Abstract. The objectives of this experiment aimed at the reaction testing of *Begonia semperflorens* cv. 'Ambassador' White minicuttings, detached from a primary culture, which was at 30 days from the plant apex of zygote seedling origin obtained by *in vitro* seed germination. Subcultivation of minipropagule of about 4-5 mm - consisting of three short petiolated leaves– was made on a solidified basic culture medium, *Murashige-Skoog* (1962), to which, besides vitamins and 30 g / l sucrose, growth regulators were also added (either 1 mg / l thidiazuron (TDZ) or 1 mg / l *indolebutyric acid* (IBA), or a mixture - equal parts - of the two growth substances). Compared with the evolution of minipropagule inoculated and cultivated on the basic medium without growth regulators, those grown on an medium containing 1 mg / l TDZ showed a very strong caulogenesis and phylogenic capacity. The counterbalance of this cytokinine with a similar amount of IBA was reduced to about half the leaves and buds generation, but stimulated calusogenesis. TDZ's total elimination from the culture medium strongly decreased the morphogenesis of *B. semperflorens* cv. 'Ambassador' White minipropagule in the subculture.
Keywords: *Begonia semperflorens*, thidiazuron, indolebutyric acid, subcultivation, micropropagation.

INTRODUCTION

Generally, the *in vitro* cultivation of *Begonia* explants was made due to the desire of cloning for commercial varieties (Ammirato et al., 1990; Rout et al., 2006).

Most research in this direction focused on the *Begonia x hiemalis* micropropagation using explants consisted from fragments of stems or leaves (Welander, 1977, 1979; Bigot, 1981, b; Reuter and Bhandari, 1981; Appelgren, 1976, 1985).

Thus, fragments of *Begonia* leaf or petiole grown *in vitro*, on culture media with cytokinines (e.g. bensiladenine - BA) adventive buds were regenerated. Out of these, excised and subcultured on media with added growth regulators, other seedlings have emerged (Chlyah-Arnason and Thanh, 1968; Maier and Sattler, 1977). But this method does not always proved to be effective, because not all the buds have the ability to regenerate plantlets (Welander, 1977, 1979; Mikkelsen and Sink, 1978; Reuter, 1980; Reuter and Bhandari, 1981; Bigot, 1981 b; Roest et al., 1981; Appelgren, 1976, 1985). Experiments have shown that juvenile plant material, compared to explants from adult plants (Olson and Walters, 1982) requires presence of a cytokinin in lower quantities in the culture medium.

In a previous study, Gergely and Cachiţă (2011) presented their results on the initiation of primary vitroculture of *Begonia semperflorens* cv. 'Ambassador' White, using caulinar apex explants taken from seedlings derived from pelleted seeds, germinated under aseptic conditions. Explants were taken from seedlings on the 30th day of its germination - under aseptic conditions – of the seeds in the sterile Petri capsules, on filter paper asepsised in the oven. They consisted of the apex *hypocotyl*, which had two cotyledons inserted on their top, and between these two the seedling buds were situated. The minicuttings of this type were inoculated on a basic culture medium Murashige-Skoog (1962) (MB - MS), modified by us and supplemented (or not) with 0.5 mg / l thidiazuron (TDZ - variant V_1) or 0.5 mg / l *indolebutyric acid* (IBA - variant V_2) or a mixture of the two growth regulators (variant V_3).

To be able to observe, as a subculture, the evolution of *Begonia semperflorens* cv. 'Ambassador' White, consisting in minipropagule, dismembered from a primary culture on the 30th day of its initiation, minipropagule - entities owned by three short petiolated leaves, deprived of any roots – there were inoculated on the above mentioned medium with TDZ or IBA, added as such or mixed with TDZ and IBA growth regulators, also used in the primary culture, but in the subculture we doubled their concentration (table 1).

MATERIAL AND METHOD

The objective of this study was to follow the reaction in vitroculture of minipropagule type explants, the formation from the leaf of the minibush separation of *Begonia semperflorens* cv. 'Ambassador' White (Fig.1), resulting from a primary culture made from apical minicuttings detached from apex seedlings obtained from seed germination under aseptic conditions of this species, on the 30th day after inoculation (Gergely and

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Cachiţă, 2011). Therefore, from a primary culture of *B. semperflorens* we detached propagules which we inoculated on Murashige-Skoog mineral culture medium (MS) (1962), with added vitamins (a mixture of: 1 mg / l thiamine HCl, pyridoxine HCl and nicotinic acid of each) the culture medium also contained 100 mg / l myo-inositol, 30 g / l sucrose and 7 g / l Difco Bacto agar. The minipropagules were harvested only from the bushes with formed leaves coming from vitrocultures made on basic mineral culture medium MB - MS supplemented with 0.5 mg / l *thidiazuron (TDZ)* in combination with 0.5 mg / l *indolebutyric acid (IBA)*. On the 30th day after the initiation of primary cultures, minicuttings regenerated from the "bush" of leaves (fig. 1 A). We detached minipropagule with a waist of about 4-5 mm (fig.1B). They were then subcultivated on variants of culture media, presented in table 1.

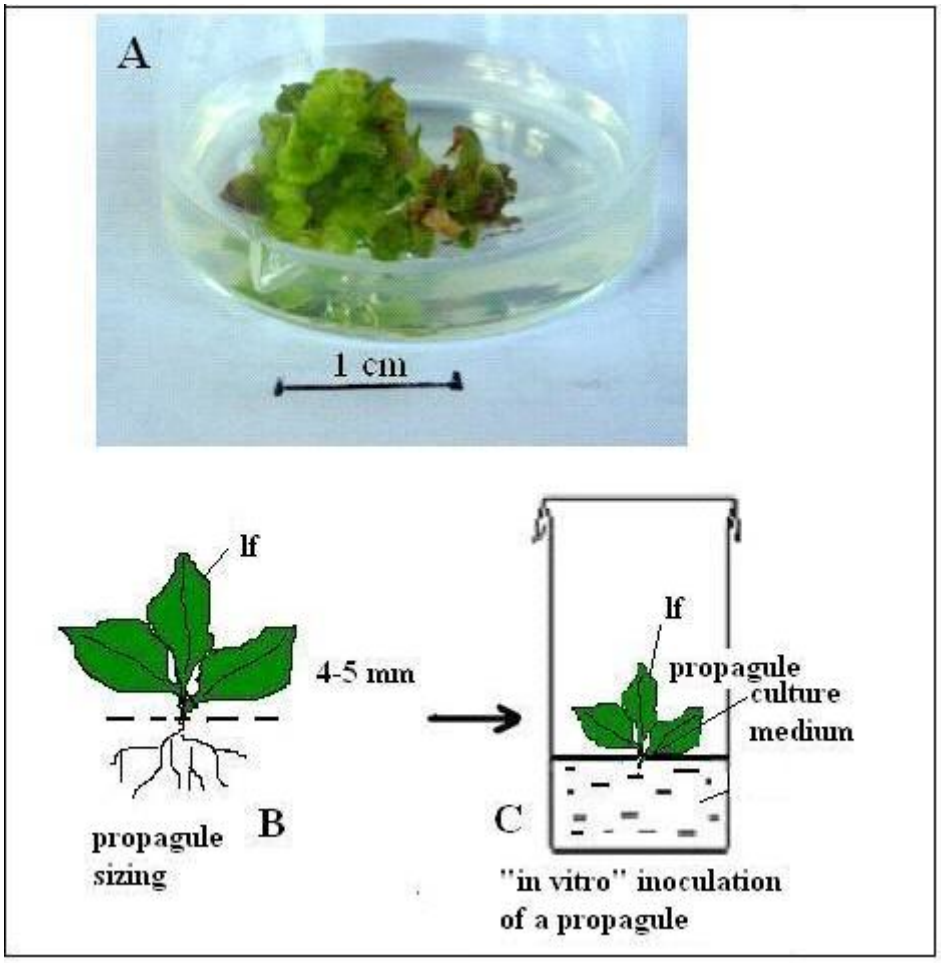

Fig. 1. Sizing of *Begonia semperflorens* cv. 'Ambassador' White minipropagule (A) initiated from apical minicuttings made from peaks of seedlings generated from germinated seeds after 30 days under aseptic conditions, on *Murashige-Skoog* basic medium (1962) (where: A - leaves regenerated from minicuttings cultivated *in vitro* for 30 days; B - dismantling primary culture in minipropagule; C - minipropagule inoculation on solidified environments BM-MS (1962).

Unlike the previous experiment in which we performed a primary culture of *Begonia semperflorens* cv. 'Ambassador' White, in the experiment presented in this paper we used the same type of culture medium - or basic Murashige and Skoog (MS) (1962), culture medium (BM) modified by us, in which we added nicotinic acid, HCl thiamine and HCl pyridoxine, 1 mg / l of each, and 100 mg / l myo-inositol, 30 g / l sucrose and 7 g / l Difco Bacto agar, and, unlike the experiments carried out in the phases in which we were to initiate a *Begonia semperflorens* cv. 'Ambassador' White primary vitroculture, during the subculture, we proceeded to increase the concentration of growth regulators introduced in the basic medium (BM) (MS) (1962) from 0.5 mg / l to 1 mg / l (table 1) pH of culture medium (prior to the autoclaving), the adjusted value was of 5.8). After the distribution of nutritional culture medium in the glass recipients (with 8 cm in height and a diameter of 3 cm) using a syringe with a capacity of 10 ml, we continued with their sterilization by autoclaving at 121 ° C for 20 minutes.

Minipropagule inoculation was made

after cooling the culture media bottles. It was conducted in laboratory, under laminar flow hood perimeter of sterile air with sterilized instruments. After inoculation, bottle filling was made with colorless polyethylene, previously sterilized with 70° alcohol, fixed with elastic. The incubation and growth of propagules was made at a light intensity of 1700 lux and with a photoperiod of 16-hour light / 8 hours dark, the ambient temperature ranged from 23° C and 27° C.

Tabel 1. Growth regulators used in culture media for cultivation *in vitro* of propagules minicuttings type of *Begonia semperflorens* cv. 'Ambassador' White.

Experimental variant code	Growth regulators introduced in culture medium (MS)*	Concentration in medium (mg/l)
V_0	Medium without growth regulators (Control group)	-
V_1'	Thidiazuron (TDZ)	1 mg/l
V_2'	Indolebutyric acid (IBA)	1 mg/l
V_3'	Thidiazuron (TDZ) + Indolebutyric acid (IBA)	1 mg/l + 1 mg/l

*MS – Murashige-Skoog medium (1962) modified by us

In the subculture experiments we used the same code to rate the alternatives, as in the primary culture (paper in print), but – as it is about doubling the amount of growth regulators, to experiment initiation of *B. semperflorens* vitrocultures – variants have been marked by an apostrophe (') (table 1).

After *30, 60* and *90 days* after propagules inoculation there were determined: their *growth in height, number and width of largest regenerated leaf, number of shoots* and *callus diameter* of phytoinocules. All data were processed mathematically and the average obtained for each parameter was reported to the parameter measured on the control group (reference group V_0), values considered as 100%.

The figure 2 illustrated an aspect of *Begonia semperflorens* cv. 'Ambassador' White vitroculture, observed at *90 days* after the minipropagules subculture operation.

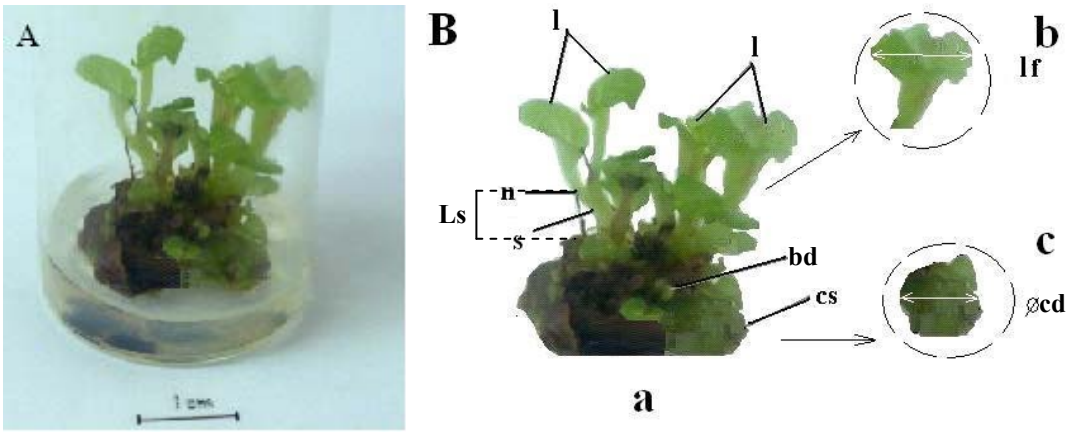

Fig. 2. Vitroculture of *Begonia semperflorens* cv. 'Ambassador' White, made from a minipropagule, at a first subculture, performed on basic mineral medium culture BM - MS (where: A - phytoinocule image in the culture container; B - same phytoinocule removed from the container, **a** - phytoinocule; s – strains; Ls-length of stem; l – leaflet; **b** - lf - large diameter of the leaf; n – node; bd – buds; cs-callus; **c** - Ø cd - callus diameter).

RESULTS AND DISCUTIONs

On the *60th day* after inoculation of *Begonia semperflorens* var. 'Ambassador' White minipropagule, on solidified medium MB-MS, both at the control of samples (V_0) (fig. 3 A) on the medium without growth regulators, and the variants V_1 '- V_3', the regeneration of buds, leaves and seedlings was observed (fig. 3 A-D), especially on variant V_1 '- MB-MS supplemented with 1 mg / l TDZ (fig. 3B), the maximum organogenesis.

As shown in figure 2 A and B at the "colony" of seedlings and leaves resulted from the subcultivated minipropagules on medium MS in the *90th-day* of vitroculture, on BM-MS layer, with added TDZ 1 mg / l (V_1'), a mass of *callus* was regenerated and it measured - on average - about 1 to 1.5 cm.

From the histograms shown in figure 4, it can be inferred that, since biomeasures made on the *30th day* after the initiation of subculture of the *B. semperflorens* minipropagules, at all evaluated parameters *(stems height, average number of leaf* / inoculum, *maximum width of the largest leaf* and *average number of buds* / inoculum), and till the *90th day* after the inoculation of them, incentive effects have dominated - the variants in culture medium that was present TDZ (V_1'and V_3'). Thus, throughout this experiment, the variant V_1', with 1 mg / l TDZ in the substrate, the four bio-measured parameters have exceeded the recorded values in the control group (V_0) (fig. 4 A-D), and *height of stems* (fig. 4 A) was higher with 190% compared with their average size to analyzed vitrocultures of the control group (V_0). *Number of neoformed leaves*, at the level of the subcultivated minipropagules was 416.2% (fig. 4 B) more than on a subculture made from a similar minipropagule inoculated and grown on MS medium without of growth regulators (variant V_0).

But not only the *number of leaves* was significantly increased on variant with 1 mg / l TDZ, but also their size, especially in terms of their width (fig. 4 C). Also a significant increase - 392% - was recorded in the *number of* present *buds* / recipient to inoculated minipropagules and grown for *90 days* on the right medium culture, variant V_1', with 1 mg / l TDZ present in the medium culture (data are provided statistically).

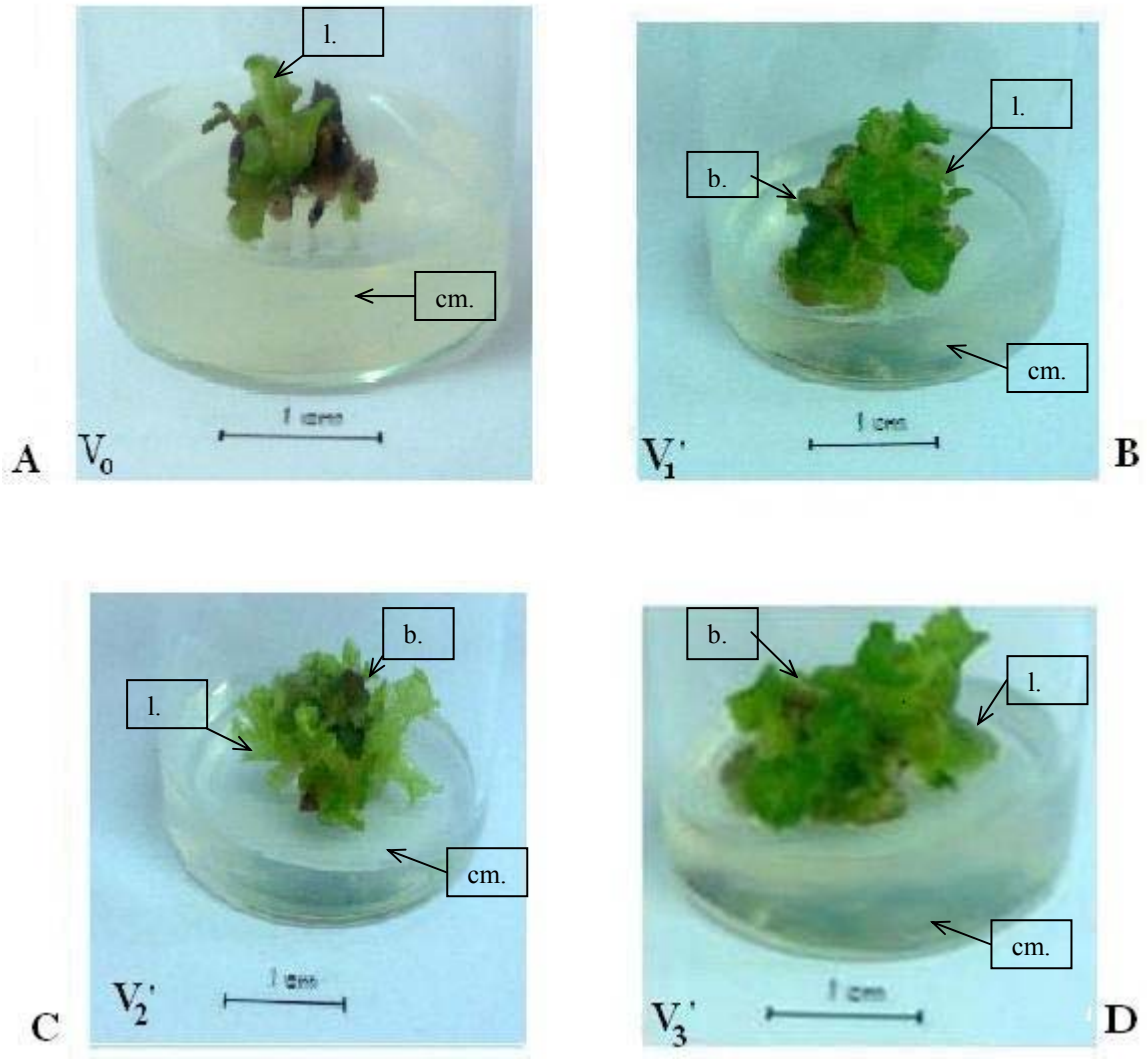

Fig. 3. Images of *Begonia semperflorens* var. 'Ambassador' White vitrocultures caught on the *60th day* of subcultivation *in vitro* of minipropagules on variants: V_0 - (control group) BM - MS without growth regulators (A); V_1'- BM - MS supplemented with 1 mg / l thidiazuron (TDZ) (B); V_2'- BM - MS supplemented with 1 mg / l indolebutyric acid (IBA) (C); V_3' - BM - MS supplemented with 1 mg / l thidiazuron (TDZ) plus 1 mg / l indolebutyric acid (IBA) (D) (where: l - leaflet, b - bud, cm - culture medium).

The presence of 1 mg / l IBA – the sole regulator of growth – in the culture medium BM-MS, compared with the recorded values in the control group (V_0), stimulation was marked, only a small extent of organogenesis (fig. 4 A-D) to that found in the control group.

The mixture of TDZ with IBA, 1 mg / l of each (variant V_3') substantially stimulated (with increases of 150%) both increased the *B. semperflorens* minipropagule stem growth and leaves neogenesis, their number being increased with 232.7%, while there was a 183.3% increase in the number of buds (fig. 4 D). Also in this variant, from the initial minipropagule, a regeneration of green *callus* in the basal zone of phytoinocules was noted.

Data obtained by us show that, as specified by Ringe (1972), TDZ – more than other cytokinines – stimulates especially the leaf and bud neogenesis.

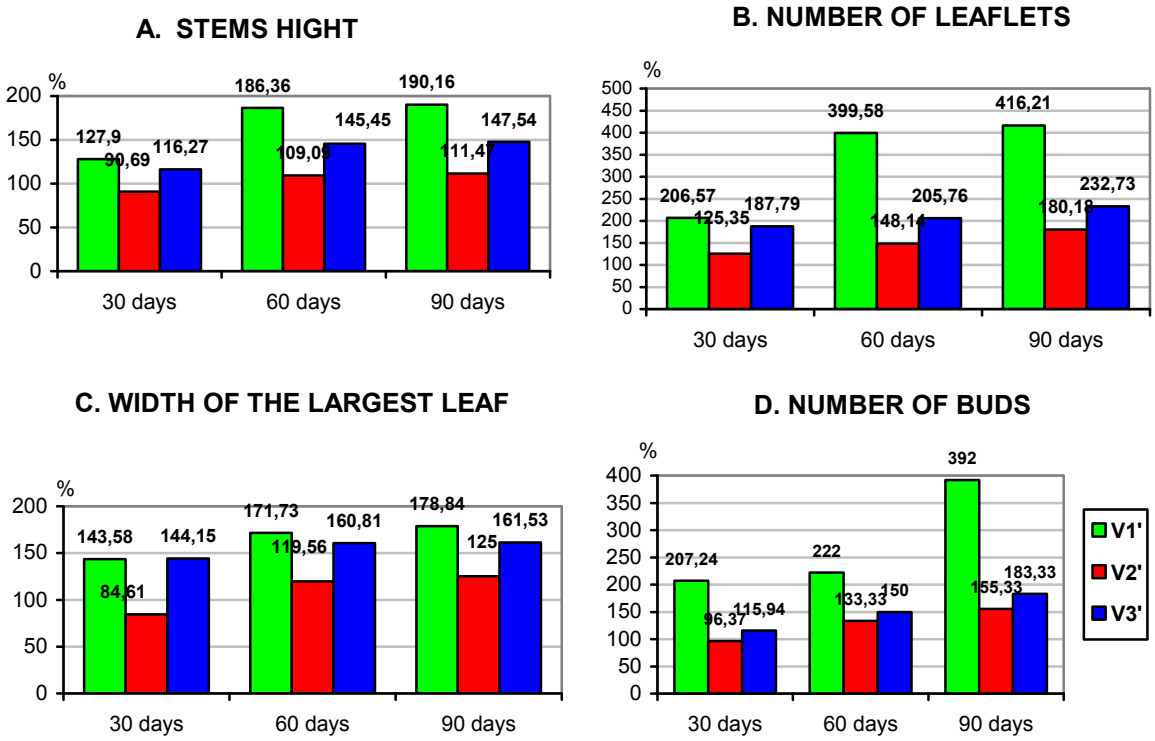

Fig. 4. Graphical presentation of average values corresponding to the parameters of the biomeasured vitrocultures of *Begonia semperflorens* cv. 'Ambassador' White in the *30th, 60th* and *90th day* after the inoculation of minipropagules on the following culture media: V_1 '- basic mineral medium culture BM-MS supplemented with the addition of 1 mg / l thidiazuron (TDZ); V_2 ' - basic mineral medium culture BM-MS supplemented with the addition of 1 mg / l indolebutyric acid (IBA); V_3'- basic mineral medium culture BM-MS supplemented with the addition of 1 mg / l thidiazuron (TDZ) plus 1 mg / l indolebutyric acid (IBA), data expressed as percentage values compared to those parameters biometrised on the control group (V_0), on which minipropagules were inoculated and grown - for *90 days* - on BM-MS medium without growth regulators; reference data considered 100%, where: A – stems height; B - number of leaf / inoculum; C - width of the largest leaf; D – number of buds / inoculum.

Thus, if vitrocultures of *Begonia* are pursuing a stimulating of minipropagule neogenesis, facilitating - through their subcultivation - an increase of efficiency in micropropagation is preferably added in MS basic culture medium by an addition of 1 mg / l TDZ. Our results contradict - at least for the subcultivated *Begonia semperflorens* minipropagules - that species of *Begonia* (after *Takayama* and *Misawa*, 1981, 1982) is an appropriate cytokinine counterbalance with an auxinic, from the culture medium.

CONCLUSIONS

1. A primary culture of *Begonia semperflorens* cv. `Ambassador` white initiated from the plant apex of zygotes origin seedlings, derived from seed germination under aseptic condition, 30 days after the start of bush with leaves and buds, regenerated from apical minicuttings dismantled in minipropagules, with a size of approx. 3-4 mm, trifoliated, short petiolated, which were subcultivated on basic solidified culture medium, Murashige-Skoog (1962), with some organic components changed by us, especially quantitatively.

2. Minicuttings of *B. semperflorens* subcultivated *in vitro* on the basic culture medium, with the addition of 1 mg / l thidiazuron (TDZ), after three months of vitrocultures, a strong stimulation of leaves and buds neogenesis and their growth have been registered.

3. The presence in the culture medium of only 1 mg / l *indolebutyric acid* (IBA) has led at the level of minipropagule type phytoinocules, to a process of morphogenesis, near the display of the recorded values in the control group, when, in the substrate culture no growth regulator was introduced.

4. The introduction of a mixture of 1 mg / l TDZ with 1 mg / l IBA in the subculture substrate of *B. semperflorens* minipropagule – made at the vitroculture level - the neogenesis of buds and leaves, but well below under the organogenesis amplitude recorded to similar inoculums grown on Murashige-Skoog basic culture medium (1962) with only the addition of TDZ at a concentration of 1 mg / l, it is also noted that under the influence of this type of hormonal balance in the basal zone of phytoinocula, the regeneration of *callus* was observed.

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