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MICROPROPAGATION OF *PHILODENDRON ERUBESCENS* WITH THE USE OF DIFFERENT BIOSTIMULATORS

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Abstract: In order to find the best type and concentration of biostimulators, *Philodendron erubescens in vitro* plants were propagated on medium with 0.1-0.8 ml l^{-1} Pentakeep-V, the same dosages of Humus FW and 1-8 ml l^{-1} Titavit (in the case of the control stock, none of them were added). After the multiplication period, plants were acclimatized in greenhouse without any biostimulator treatment (as substrate, white peat and vermiculite mixture in 2:1 ratio was used). In general, most of the *in vitro* plants' survival ratio was higher than 80-85%, and 2-4 ml l^{-1} Titavit and 0.2 ml l^{-1} Humus FW effected the best values (95-100%); however, the latter product resulted the fewest shoot and root, the shortest leaves and roots, and the lowest leaf chlorophyll (a+b) contents. Pentakeep-V was suitable for faster shoot development and optimal plant elongation, while Titavit rather stimulated rooting and enhanced leaf pigment level, but only in lower concentrations, because the highest dose decreased all plant parameters.

Keywords: Philodendron, biostimulator, plant parameters, multiplication, acclimatization

1. Introduction

There are almost 500 *Philodendron* species belonging to the family Araceae (Boyce and Croat, 2012), and due to their attractive, various sized and coloured leaves, most of them are popular indoor pot plants. However, micropropagation of Philodendrons is a common practice, especially in the case of some species that propagated more slowly by cutting (Tillyné and Honfi, 2008), relatively few of them multiplied in this way. Mostly, micropropagation procedures and data were reported in the case of not climbing or short-stemmed taxa such as *P. tuxtlanum* (Jámbor-Benczúr and Márta-Riffer, 1990), *P. cannifolium* (Han and Park, 2008), *P. erubescens* varieties (Fahmy et al., 1998; Chen et al, 2012) and *P. bipinnatifidum* (Alawaadh et al., 2020). According to these studies, sterilized explants (lateral of apical buds) were collected from mother plants and newly developed shoots were multiplied or rooted on Murashige & Skoog (1962) media containing various cytokinins and auxins. As final step, acclimatization was carried out gradually (duration time: some weeks, according to the species), usually on sterile substrate (mixtures of peat, vermiculite and perlite) with 80-100% survival.

Although the culture media that generally used for *in vitro* propagation consist of artificial (and chemically clearly defined) ingredients, in order to get better results (or because of the environmental, economic regulations), natural plant-derived products have also been tested with more or less success (Jámborné and Dobránszki, 2005; Molnár et al., 2011).

In more details about the use of biostimulators, the 5-amino-levulinic acid (ALA)-containing chlorophyll precursor (Vágújfalvi, 2007) Pentakeep-V was less effective for Hosta 'Gold Drop' *in vitro* cultures; because it generated callus malformation which totally reduced shoot and root production. Furthermore, Pentakeep-V also negatively affected acclimatization, resulted weaker plants with lower survival (Ördögh et al., 2019). On the other hand, this product increased (mainly at a dose of 0.8 ml l^{-1}) the leaf chlorophyll contents and shoot development of *Sorbus borbasii* 'Herkulesfürdő' *in vitro* plants (Vidák, 2014).

Though titanium ascorbate is not an organic originated complex molecule, when dissolved in water, it can be absorbed by plants directly and enhances the absorption of other nutrients (Lyu et al., 2017). It was also marketed as Titavit, and Pais (1983) noticed 10-20% yield increasing, summarizing the results of domestic plant experiments. Adding this preparation to a sterile culture medium at concentrations of 0.1-0.5-1-2 ppm, the two lower doses resulted better germination rate (95% instead of 5%) during *in vitro* sowing of *Escobaria* cacti, and reduced germinated specimens' vitrification (Szabó and Tillyné-Mándy, 2007). In the case of *Hosta* 'Dew Drop', 0.5 mg l^{-1} Titavit increased the number of shoots by 30% compared to the control (the leaves' chlorophyll level also was the highest at this time). In addition, higher antioxidant content observed in the roots and the leaves; so, Titavit exerted a stress-reducing effect (Tillyné Mándy et al., 2007).

For another *Hosta* variety ('Gold Drop'), this product did not significantly affect the shoot number and leaf length, but all shoots became longer by 24.8-32.2% based on the doses used (0.5-10 mg l^{-1}). The treatment primarily stimulated root formation: compared to the 15% rate experienced in the control group, every concentration resulted 100% rooting, and the number of roots approximately tripled, although a decrease occurred with increasing the dose, just like when a huminite extract, Humus FW was used. The addition of 1-2 ml l^{-1} of the latter substance raised it to 65%, while 3-4 ml l^{-1} reduced rooting (to 30%), and the number and length values of the roots were significantly lower than in the case of Titavit. At the same time, the average shoot number of the Humus-treated plant increased 4-5 times compared to the specimens grown on Titavit-supplemented medium, and it had a positive effect on the leaf length (Ördögh et al., 2010). Enhanced rooting was achieved in apple cultures on media with 0.5-1.5 ml l^{-1} Humus FW, only the highest level (2 ml l^{-1}) proved to have an inhibitory effect when the number of rooted specimens decreased (Dobránszki et al., 2010). 1.5 ml l^{-1} Humus also significantly increased (almost doubled, from 55 to 94%) the ratio of rooted *Sorbus rotundifolia* 'Bükk szépe' plants, with an average root number increasing from 3.1 to 9.3 (Dobránszki et al., 2012).

Summarizing, the optimal type and concentration of biostimulators were based on the plant species or varieties. In this study, the aim was to determine optimal concentration of Pentakeep-V, Titavit and Humus FW for the *in vitro* propagation of *Philodendron erubescens.* The aftereffect of these products was also investigated during the acclimatization.

2. Materials and methods

2.1. Plant material, culture conditions

The basis of the research was an *in vitro* culture of *Philodendron erubescens* maintained in the laboratory of the Department of Floriculture and Dendrology. During multiplication, I divided the plant clumps into separate shoots and transferred them into Erlenmeyer flasks, filled with S-medium containing BM (Jámbor-Benczúr and Márta-Riffer, 1990) macroelements and Heller (1953) microelements, supplemented with Titavit (1, 2, 4 and 8 ml l^{-1}), 0.1, 0.2, 0.4 and 0.8 ml l^{-1} Pentakeep-V or the same concentration of Humus FW. No hormones (auxins, cytokinins) were added to the media (and as control: no biostimulators). I kept the cultures in the light room of the laboratory under 16-hour illumination with cold and warm white fluorescent lamps (Polylux XLR FT8/30W/830 and 860, USA), at an average temperature of 20-25 °C. As acclimatization (in one of the greenhouses of our department), all specimens were placed in 3x3 cm rectangular plastic pots filled with 2:1 mixture of white peat and vermiculite, and 5 months later, I transferred them into 10x10 cm plastic pots filled with the same substrate. During this stage, plants were not treated by any of the biostimulators. Survived *in vitro* and acclimatized Philodendrons (40 specimens per treatment) were examined according to the parameters shown in chapter 2.2.

2.2. Examined plant parameters, data and statistical analysis

Survival ratio (%) of *in vitro* and acclimatized plants was determined, additionally, shoot and root number, plant height, the length of the longest leaf and root were recorded. In addition, I collected 4 x 100 mg chopped leaf samples from each culture for spectrophotometric analysis of chlorophyll concentration, calculated by formula according to (1).

$$\text{Chlorophyll (a+b) content } (\mu g\ g^{-1}) = (20.2 \times A644 + 8.02 \times A663) \times V/w \quad (1)$$

where:
V= volume of tissue extract (10 ml)
w= fresh weight of tissue (0.1 g)
A= absorbance (Arnon, 1949).

All data were evaluated with the use of SPSS 23.0 (IBM Corp., USA) and analysis of variance was conducted to determine the statistical significance between the treatments. In the cases of significant differences, the means separated by Tukey's test at $p \leq 0.05$.

3. Results

The *in vitro* plants' survival rate was 95% or higher in the control group and on media containing 2 or 4 ml l^{-1} Titavit and 0.2 ml l^{-1} Humus FW (hereinafter: Humus). In the presence of Pentakeep -V (hereinafter: Pentakeep), the dose of 0.4 ml l^{-1} gave

the maximum value (90%). The highest concentrations of all biostimulators resulted reduction, especially when Humus was used. During the acclimatization phase, additional specimens perished, this loss was more than 10% in the stocks from the medium supplemented with 0.4 ml l^{-1} Pentakeep and 0.2 ml l^{-1} Humus. In the case of Titavit, the ratio remained unchanged at almost all concentrations, thus, every *in vitro* plants stayed alive later, in the whole acclimatization period.

3.1. Shoot number

Most cases (and compared to the control), biostimulators not effected significantly more shoots during the in vitro multiplication stage (and later, neither in the acclimatized stocks). 0.2 and 0.4 ml l^{-1} Pentakeep resulted the most sprouts (1.5 shoots in vitro, and 2.38-2.45 pieces during the acclimatization), although, almost all Humus treatments proved to have a negative effect in both stages: this preparation resulted only 1.03-1.13 shoots. In several cases (control, 1 ml l^{-1} Titavit, 0.1 ml l^{-1} Humus, and all media containing Pentakeep), the plants' shoot number doubled in the acclimatization phase.

3.2. Plant height

The *in vitro* stocks did not show significant differences, compared to the control's average of 28.76 cm, only 4 ml l^{-1} Titavit and 0.4 ml l^{-1} Pentakeep considerably increased the height to around 36 mm. The acclimatized plants tripled or quadrupled their height in most groups; they reached or exceeded 90 mm in the majority of cases. As compared to the control value (92.17 mm), the difference proved to be significant when 2 ml l^{-1} Titavit (139.38 mm), all Pentakeep doses (102.85-141.58 mm) and higher levels of Humus previously used during multiplication. The latest product reduced the plant height below 70 mm.

3.3. Leaf length

The largest leaves of the *in vitro* plants did not exceed 35 mm either (mainly in the control and Humus-treated groups), although statistically significant differences were not detected mostly. The only exceptions to this (and compared to the untreated group) were the values of 34.03-34.68 mm resulted by 2 and 4 ml L^{-1} Titavit and 0.4 ml l^{-1} Pentakeep. During acclimatization, the differences between the groups became more pronounced; three concentrations each of Titavit and Pentakeep led to the development of leaves with significantly longer sizes (generally more than 110 mm), while as a negative aftereffect of Humus, they became significantly shorter, usually below 80 mm.

3.4. Root number

The root number of the *in vitro* plants grown on medium with 4 and 8 ml l^{-1} Titavit was significantly higher (9.42 and 9.67 pieces) compared to the average of 8.1 obtained in the control group. The other treatments resulted fewer roots in the majority of cases, especially in the presence of Pentakeep (5.06-6.7 pieces). The root system of the acclimatized plants were partially damaged. This is normal, because *in*

vitro roots are usually weak and dysfunctional (do not survive acclimatization), but new ones will be formed soon. It follows that reduced values were observed in the acclimatized stocks; in some cases (control, 4 and 8 ml l^{-1} Titavit, and 0.1 -0.4 ml l^{-1} Humus), the root number was reduced by about half. As aftereffect, 1 and 2 ml l^{-1} Titavit proved to be the best: on average, 5.79 and 6 roots formed.

3.5. Root length

In the *in vitro* plant cultures, compared to the control average of 42.18 mm, almost all Titavit and Pentakeep dosages markedly increased the length of the longest roots (up to around 60 mm in several cases). At the same time, Titavit led to a continuous decrease with increasing concentration (as an aftereffect also, in the acclimatized plants). Raising the Humus dose had similar negative effect on the root length: this product resulted the shortest roots. Regardless of the treatment, the average root length tripled or quadrupled in the acclimatized plants, and the highest values were obtained in the stocks originated from the media supplemented with the lowest concentration of Titavit (213.26 mm) and Pentakeep (193.97 mm).

3.6. Chlorophyll (a+b) content

The *in vitro* plants's chlorophyll values did not differ, excepting the effect of 4 ml l^{-1} Titavit, which significantly enhanced this parameter (up to 1378.7 μg g^{-1}). The same effect observed in the acclimatized stocks (as an aftereffect of 4 ml l^{-1} Titavit, the highest level was 3181.5 μg g^{-1}), and difference was also significant in accordance with 0.4 ml l^{-1} Humus (resulted the lowest average: 1515.8 μg g^{-1}). The chlorophyll content of the acclimatized plants roughly tripled compared to the *in vitro* formula or equation.

4. Discussion

The highest *in vitro* survival was recorded when 2 or 4 ml l^{-1} Titavit was used, whereas the highest concentration (for all biostimulators) led to a decrease. During acclimatization, the loss was the smallest in the case of plants grown on Titavit-containing mediums, all individuals survived. The biostimulators had no significant effect on shoot formation, but Humus proved to be the least stimulating, while Pentakeep was the best. Considerable plant height differences were shown only between the acclimatized groups and the plants that came out of the *in vitro* life stage usually became three-four times taller in this last micropropagation phase. Among the biostimulators, Humus resulted the lowest, and Pentakeep the highest plants. During acclimatization, the most concentrated doses reduced their height as an aftereffect, especially in the case of Humus. In terms of the leaf length, significant differences were detected principally in the acclimatized stocks, and the size of the leaves usually tripled compared to the *in vitro* specimens, with the exception of Humus, which resulted the smallest averages. *In vitro* plants had more roots (mainly effected by 4 and 8 ml l^{-1} Titavit), but in acclimatized plants, sometimes half of their roots died. The root number was the lowest on media with Humus in almost all doses, while 1 and 2 ml l^{-1} Titavit had positive aftereffect in the acclimatization phase.

Similar tendency was observed in the case of the roots' length. Evolved roots (that became at least three to four times longer in the course of the acclimatization) were the longest when the lowest dose of Titavit and Pentakeep were used, and Humus had the least effect. The chlorophyll (a+b) content of acclimatized plants also trebled compared to their previous *in vitro* stage; 4 ml l^{-1} Titavit effected significantly the highest, and Humus eventuated the lowest pigment levels (mainly in higher doses).

Overall, biostimulators had strong aftereffects during the acclimatization, great differences were shown especially compared with the control. Titavit was the most effective, and Humus proved to be the least suitable, offered only in lower doses.

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