

The effect of different biostimulators on morphological and biochemical parameters of micropropagated *Hosta* 'Gold Drop'

Ördögh, M., Beregi, Zs. & Tillyné Mándy, A.

Department of Floriculture and Dendrology, Faculty of Horticultural Science, Szent István University,
H-1118 Budapest, 35-43. Villányi str., Hungary,
Author for correspondence: ordogh.mate@kertk.szie.hu

Summary: During *in vitro* multiplication of *Hosta* 'Gold Drop', 20 g l⁻¹ sucrose, 5.5 g l⁻¹ agar and 4 concentrations (0.1-0.8 ml l⁻¹) of Ferbanat L, Kelpak, Pentakeep-V were added to half-strength Murashige and Skoog (MS) basal medium. As compared to the control and other biostimulators, plants with lower peroxidase activity, larger fresh weight, more, longer shoots and roots, larger leaves were developed on medium containing Kelpak. The best concentration was 0.4 ml l⁻¹ for *in vitro* rooting, shoot formation, plant weight and ex vitro chlorophyll, carotenoid level, peroxidase activity. Pentakeep was the less efficient biostimulator, increasing of its concentration mostly decreased root and shoot values (furthermore, abnormal callus formation was observed, as non-wanted effect), chlorophyll content and sizes (length, width) of leaves, not only during *in vitro* propagation but also (as after-effect) acclimatization because of the high mortality and weakly developed survivor plants.

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Introduction

In order to eliminate harmful environmental effects, considerable limitations have been placed on several compounds applied in horticultural production, which consist synthetic growth regulators (e.g. auxins) and plant protection chemicals (Ludwig-Muller, 2000). Therefore, environmentally friendly, substitutive formulations such as biostimulators (which can be used only under definite, accurate registration requirements) are often come into focus (Dąbrowski, 2008) and certain preparations, such as Kelpak, Pentakeep-V might be an alternative to chemicals banned by EU (Dobrzański et al., 2008). Biostimulators contain different growth regulators or other hormonal components. These natural, organic preparations often decrease stresses, increase physiological activity, accelerate root development and regularly applied in horticulture but rarely in plant propagation (Szabó et al., 2011).

Positive results during ornamental floriculture application of relevant biostimulators (Pentakeep-V, Kelpak, Ferbanat L) have already reported. The chlorophyll-precursor (Vágújfalvi 2007; Kosáry, 2008) 5-amino-levulinic-acid (ALA) containing Pentakeep-V resulted more lateral shoots of *Tillandsia usneoides* when optimal concentration (0.5 ml l⁻¹) was used (Tilly-Mándy et al., 2010a). In the case of growing *Petunia* cv. Veranda 'Rose Vein' seedlings, compact plants with smaller leaf-area, earlier flowering time and more flowers were produced in the presence of 0.3 ml l⁻¹ Pentakeep-V (Duchaj, 2011). The same (and higher: 0.5 ml l⁻¹) dose was optimal for *Saintpaulia ionantha* (Tilly-Mándy et al., 2010b), because treated plants produced flowers two weeks earlier than the control and developed larger leaf-rozettes with higher chlorophyll concentration. Similarly, *Begonia x tuberhybrida*

'Nonstop' plants' growing time was shortened, and the chlorophyll contents of their leaves was higher especially when 0.5 ml l⁻¹ Pentakeep-solution was sprayed (Kisvarga et al., 2015). Awad (2008) also experienced that the use of 0.04 or 0.08% Pentakeep-V resulted more chlorophyll and faster growing of *Phoenix dactylifera* 'Kalas': treated specimens got the value for marketable condition 4-5 months sooner than by the control plants. If *Pelargonium zonale* 'Serena' cuttings were soaked or soaked + sprayed with 0.5% Pentakeep-V, significantly longer, more and heavier roots were formed, but no significant differences were obtained between the way of biostimulator application (Köbli et al., 2012). Compared to the untreated groups, treatment significantly increased the bulb yield (bulb number and size) of *Tulipa* varieties 'Leen van der Mark' and 'Ballerina' (Yoshida et al., 2006). 0.03 or 0,05% Pentakeep-V gave the best results (heavier, more corms or bulbs) of other bulbous ornamental cut flowers like *Allium christophii*, *Tulipa* 'Lucky Strike', *Lilium* 'Star Gazer' and *Gladiolus* 'White Friendship' (Krzyszewska, 2007). With the use of Pentakeep-V during cut flower production of *Lilium* cultivars (Abay, 2014), higher chlorophyll content, better vegetative developments, enhanced CO₂ assimilation were detected.

Another seaweed product Kelpak (in dilution 0.2%) positively influenced the growth of *Sorbus aucuparia* seedlings, their plant height, root and caliper characteristics were significantly higher (Magyar et al., 2008). The same treatment increased the number and weight of shoots of *Prunus* Marianna 'GF 8-1' and *P. mahaleb* 'Bogdány' stockplants. In case of 'GF-8-1' cultivar, improvement of leaf chlorophyll

content was also detected (Szabó & Hrotkó, 2009). Furthermore, application of this biostimulator on 'GF-8-1' stockplants resulted the highest ratio of rooting and enhanced the fresh weight of cutting during rooting in comparison with non-treated (control) cuttings (Szabó et al., 2011), and higher rooting rate of *Prunus mahaleb* 'Magyar' (a difficult-to-root cultivar) cuttings was obtained (Szabó, 2015).

The use of Ferbanat L (also known as Bistep) and Kelpak equally increased root weight, but further Ferbanat treatment (in 0.2 and 0.4 % concentration) resulted longer stems and significantly larger flower buds of *Lilium* oriental hybrid cv. 'Rialto' (Tilly-Mándy et al., 2012; Takács et al., 2015). During growing of *Petunia x grandiflora* 'Musica Blue' seedlings, 0.1% Ferbanat L improved shoot development, and further growth was achieved in the presence of higher (0.2 or 0.3%) doses (Kisvarga et al., 2014). For container growth *Forsythia x intermedia* 'Beatrix Farrand' plants, 0.5% solution was the best because of the larger root and shoot weight, longer shoots with more, larger, thicker leaves (Kovács et al., 2017).

In order to successful *in vitro* propagation of different plants, wide variety of natural, phytochemical substances can be used (Jámborné & Dobránszki, 2005). Although chemically defined accessories are generally used to the media, sometimes, organic supplements are optional and useful (Molnár et al., 2011). Few cases, Kelpak, Ferbanat L, Pentakeep-V biostimulators were used as ingredients of *in vitro* media.

Kelpak is a brown alga (*Ecklonia maxima*) extract, prepared by a cell burst process and its active components were suggested to be auxins and cytokinins (Featonby-Smith and Van Staden, 1984). During *in vitro* propagation of potato cv. 'BP1' (Kowalski et al., 1999), results shown that the use of this easy-to-apply, cheap product rejuvenated all plantlets, but higher Kelpak concentration (0.5 and 1 %) decreased shoot length, fresh weight and reduce root development; thus, optimally lower (0.25%) dose of Kelpak eventuated larger plants with well-developed rootage. More roots, higher chlorophyll content, heavier *in vitro* *Melissa officinalis* plants were found in the presence of this seaweed product (1% was optimal dose), and for best proliferation of potatoes, higher (1.5%) concentration was recommended (Tantos, 2002). *Sorbus borbasii* 'Herkulesfürdő' plants required lower Kelpak levels (0.1 or 0.2 ml l⁻¹ instead of 0.4, 0.8 ml l⁻¹) in favour of sufficient shoot formation and higher chlorophyll, but no roots developed with the use of any quantity of this biostimulator (Vidák, 2014).

The application of 0.1 ml l⁻¹ Ferbanat L decreased peroxidase enzyme activity, and higher dose (0.5 ml l⁻¹) increased root length, number of new leaves and chlorophyll contents of *in vitro* *Myrmecophyla tibicinis* and *Peristeria elata* orchid plants culturing on modified MKC (Knudson, 1946) basal medium. The highest level (1 ml l⁻¹) of this agent resulted slower, weaker growing, lower chlorophyll and higher peroxidase values (Thuróczy, 2012). In contrast, the most concentrated Ferbanat L solution (0.8 ml l⁻¹) resulted the highest chlorophyll, carotenoid content and the lowest peroxidase enzyme activity of *in vitro* *Sorbus borbasii* 'Herkulesfürdő' plants. For this Hungarian sorb cultivar, Pentakeep-V has similar positive effect on the same physiological features (and additionally, on the number of shoots, size of leaves) especially with the application of the highest (0.8 ml l⁻¹) dose (Vidák, 2014). The same (and half-strength, 0.4 ml l⁻¹) concentration of this product resulted the highest number of *in vitro* developed shoots and the longest leaves (with the highest chlorophyll content) of *Philodendron*

erubescens, however, the higher concentration of Pentakeep-V decreased the length of roots. Additionally, acclimatized *Philodendron erubescens* plants grown higher and developed longer leaves when previously cultured them on medium contained Pentakeep-V comparing the control (Asztalos, 2014).

Plantain lilies (members of the genus *Hosta*, belonged to the *Asparagaceae* family) are popular, shade tolerant East-Asian (mainly Japanese) ornamental perennial plants with robust rhizome, variable leaves and attractive, white or violet-blue, bell-shaped, summer flowers with low pollen, total sugar mass production (Bozek et al., 2015). *Hosta* propagation by seeds or division are conventional but relatively unproductive methods with heterogeneous, slow growing seedlings and/or low multiplication ratio (Hamrick, 2003; Rice, 2006). If large-scale production of pathogen-free, uniform, high-quality plants is required, micropropagation is economical and efficient (Jámborné and Dobránszki, 2005). Several *Hosta* cultivars were efficiently *in vitro* cultured on Murashige and Skoog (MS, 1962) basic medium, and type or concentration of plant hormones was often depended on the cultivars. As example, 6 mg l⁻¹ benzyle-aminopurin (BAP) was optimal for *H.* 'Devon Green', *H.* 'Blue Cadet', *H.* 'Samurai', while *H.* 'Gold Haze', *H.* 'Gold Drop' needed lower dilution, only 3 mg l⁻¹. As sourcing carbohydrates, 20-35 g l⁻¹ sucrose is recommended, accordingly to the cultivars (Szafián, 2010). Sometimes, agar-free, liquid media resulted more *in vitro* shoots, roots and greater dry weight (Adelberg et al., 2000; Adelberg, 2005).

For our study, we chose an easy-to-care, trouble free cultivar as test plant, *Hosta* 'Gold Drop'. Previously, a similar (but white-patterned) cv. *Hosta* 'Dew Drop' was successfully micropropagated by *in vitro* application of Pentakeep-V, Ferbanat L and Kelpak (instead of different auxins and/or cytokinins). These biostimulators effectively improved the number and fresh weight of *in vitro* grown shoots and (as after-effect) acclimatized plants usually developed larger leaves with higher chlorophyll and carotenoid contents especially when Ferbanat L or Kelpak was previously applied during *in vitro* propagation (Gere, 2017). Due to these positive results, we wanted to know whether *Hosta* 'Gold Drop' gives similar or different reactions if we use the same type and concentration of biostimulators.

Materials and methods

Origin of plant material

The experiments were carried out with the use of rootless, 2.5-3 cm sized, 3 leaved shoots as explants originated from *in vitro* stock of *Hosta* 'Gold Drop', a dwarf-sized cultivar with small, pale, yellowish-green leaves and violet flowers (Schmid, 1991; Liu and Zhao, 2012). The *in vitro* and acclimatization studies were done in the laboratory and greenhouse of the Department of Floriculture and Dendrology, Szent István University.

Culture establishment

For *in vitro* culturing, rootless shoots with 3-4 leaves (as explants) were planted on Murashige and Skoog (MS, 1962) basal medium with half-strength macroelements as control and four different concentrations (0.1, 0.2, 0.4, 0.8 ml l⁻¹) of three biostimulators, Ferbanat L (originally made by the Turkish Ekosistem company, in Hungary, it was allowed as „Bistep

plant conditioner”), Kelpak (Kelp Products Ltd., South Africa) and Pentakeep-V (Cosmo Seiwa Agriculture Ltd., Japan). The control did not contain biostimulator at all. Every medium contained 20 g l⁻¹ sucrose (Reanal Finomvegyszergyár Zrt., Hungary) and 5.5 g l⁻¹ agar (Sigma-Aldrich, Merck, USA). The pH was adjusted to 5.6 with KOH and autoclaving was done for 35 minutes on overpressure (10⁵ Pa). *In vitro* cultures were maintained at 22 ± °C under 16/8 photoperiod with a photosynthetic photon flux density of 40 μmol m⁻¹ s⁻¹. 14 weeks later, *in vitro* morphological data (shoot and root number and length, leaf length and width, fresh plant weight) were recorded.

For determining the after effect of examined biostimulators *in vitro* grown plants from every treatment were transplanted into a soil mix of 50% perlite + 50% peat as acclimatization in greenhouse conditions (70% relative air humidity, 20-25 °C, without artificial lighting and fertilization). Worthy of note: the use of *in vitro* rooting medium (containing different types or concentrations of auxins) was not necessary before acclimatization, because we observed spontaneous root development during *in vitro* *Hosta* ‘Gold Drop’ culturing on all medium with or without biostimulators. Twenty weeks after the start of acclimatization, *ex vitro* shoot and leaf parameters namely number and length of shoots, length and width of leaves were measured (plant weight, roots were not examined in order to avoid plant’s injuries). Additionally, rate of abnormally developed *in vitro* plants (with callus) and survival of *ex vitro* (acclimatized) specimens were determined. Important to note that only the after-effects of *in vitro* applied biostimulators were studied in the stage of acclimatization, that is why uniform soil mix was used for every plant specimen. Each experiment was repeated twice and 30 plants per treatment was examined.

Measurement of biochemical parameters

For determination of chlorophyll a + b (thereinafter: chlorophyll) and carotenoid content, 100 mg leaf sample was used from both *in vitro* and acclimatized plants, three fold per treatment. Leaves were homogenized by a dash of quartz sand and 10 ml acetone (80%). After 24 hour cooling on +4 °C absorbance of solution was measured by GeneSys VIS-10 (Thermo Fisher Scientific Inc., USA) spectrophotometer at 644, 663 and 480 nm wavelength. Chlorophyll and carotenoid contents (μg g⁻¹) were calculated by formula (20.2 × A₆₄₄ + 8.02 × A₆₆₃) × V/w and (5.01 × A₄₈₀)/w; where V= volume of tissue extract (10 ml), w= fresh weight of tissue (0.1 g), A= absorbance (Arnon, 1949).

In the case of assaying peroxidase (POD) activity, 3×150 mg leaf (from *in vitro* and *ex vitro* plants) per treatment were homogenized in a refrigerated mortar with the use of 1.5 ml KH₂PO₄ (pH=6.5, 0.05 M). After centrifuging (4 °C, 20 minutes, 13500 rpm), separated extracts (without solid particles) were used for spectrophotometric investigations (adjusted wavelength: 460 nm). For reaction, plant-extracts (3 × 0.01 ml/treatment) were mixed with 1.7 ml C₂H₃NaO₂ (pH=4.5, 0.1 M), 0.03 ml H₂O₂ and 0.02 ml ortho-dianisidine (3, 3'-dimethoxybenzidine) as chromogen reagent. Enzyme activity (U mg⁻¹) was calculated with formula (ΔA1 × attenuation)/ε; where ΔA1 = absorbance change/1 min, ε = 11.3: extinction coefficient of ortho-dianisidine (Shannon et al, 1966; Blinda et al., 1996).

Three repetitions from every treatment was used for examinations of all biochemical parameters.

Data and statistical analysis

Data (chlorophyll and carotenoid content, POD activity, length and width of leaves, number and length of shoots and roots, fresh weight of plants) were evaluated by SPSS 23.0 (IBM Corp., USA). An analysis of variance (ANOVA) was conducted to calculate the statistical significance of all data presented. When significant differences between treatments were found, the means were separated by Tukey’s and Games-Howell’s test at p ≤ 0.05.

Results and discussion

Number and length of roots

In vitro root number and length were the highest on medium supplemented with 0.1 ml l⁻¹ Ferbanat (32.13 and 83.93 mm), or 0.4 ml l⁻¹ Kelpak (34.23 and 78.2 mm, **Figure 1 A**). More concentration of Pentakeep eventuated less and shorter roots, significantly the lowest values were observed when 0.8 ml l⁻¹ Pentakeep was used (**Figure 1 B, Table 1**). Direct proportion was experienced between root number and length. In another trial (*Hosta* ‘Dew Drop’ micropropagation - Gere, 2017), the longest and the highest number of roots were achieved on medium supplemented with 0.1, 0.2 ml l⁻¹ Pentakeep (26.76 and 24.55 root) and 0.2, 0.4 ml l⁻¹ Ferbanat (88.47 and 81.88 mm). Increasing of Pentakeep concentration decreased the number and length of roots, similarly to our research.

Table 1. Root number and length, fresh weight of *in vitro* grown *Hosta* ‘Gold Drop’ plants cultured on Murashige and Skoog (1962) medium with 0.1-0.8 ml l⁻¹ Ferbanat L, Kelpak, Pentakeep-V. Data represented by mean ± standard deviation (SD). Means with different letters are significantly different according to Tukey’s and Games-Howell’s comparison test at p<0.05

		Root number ± SD	Root length (mm) ± SD	Fresh plant weight (g) ± SD
Control		31.48 ± 7.86 efg	83.25 ± 34.87 e	1.5 ± 0.82 bc
Ferbanat L (ml l ⁻¹)	0.1	32.13 ± 6.35 fg	83.93 ± 29.61 e	1.48 ± 0.57 bc
	0.2	26.86 ± 5.44 cde	82.1 ± 36.82 e	1.31 ± 0.66 bc
	0.4	23.8 ± 3.33 bc	51.73 ± 17.06 b	1.33 ± 0.4 bc
	0.8	25.46 ± 6.06 cd	59 ± 31.4 bcd	1.43 ± 0.44 bc
Kelpak (ml l ⁻¹)	0.1	28.86 ± 5.65 def	62.93 ± 23.91 bcde	1.68 ± 0.61 bc
	0.2	33.1 ± 4.89 fg	77.1 ± 23.63 cde	2.26 ± 0.65 de
	0.4	34.23 ± 5.41 g	78.2 ± 29.21 de	2.53 ± 0.95 e
Pentakeep-V (ml l ⁻¹)	0.1	27.17 ± 4.75 cde	55.75 ± 19.44 bc	1.44 ± 0.74 bc
	0.2	22.75 ± 5.38 bc	49.58 ± 15.4 b	1.14 ± 0.56 ab
	0.4	20.37 ± 6.13 b	41.75 ± 20.16 b	1.17 ± 0.48 abc
	0.8	5.07 ± 3.12 a	7.6 ± 5.47 a	0.7 ± 0.52 a

Fresh plant weight

Kelpak in every concentrations resulted the heaviest *in vitro* plants, especially in the case of 0.2 and 0.4 ml l⁻¹, when significantly higher values (2.26 and 2.53 g) were obtained. It is worth notify that the latter treatment also effected the most and longest shoots and the lowest leaf, root averages were achieved on medium with Pentakeep. During *in vitro* propagation of *Hosta* ‘Dew Drop’ (Gere, 2017), 0.2 and 0.4 ml l⁻¹ Ferbanat was the best for fresh weight (2.62 and 2.93 g), furthermore, the same treatments enhanced shooting, shoot and root length principally.

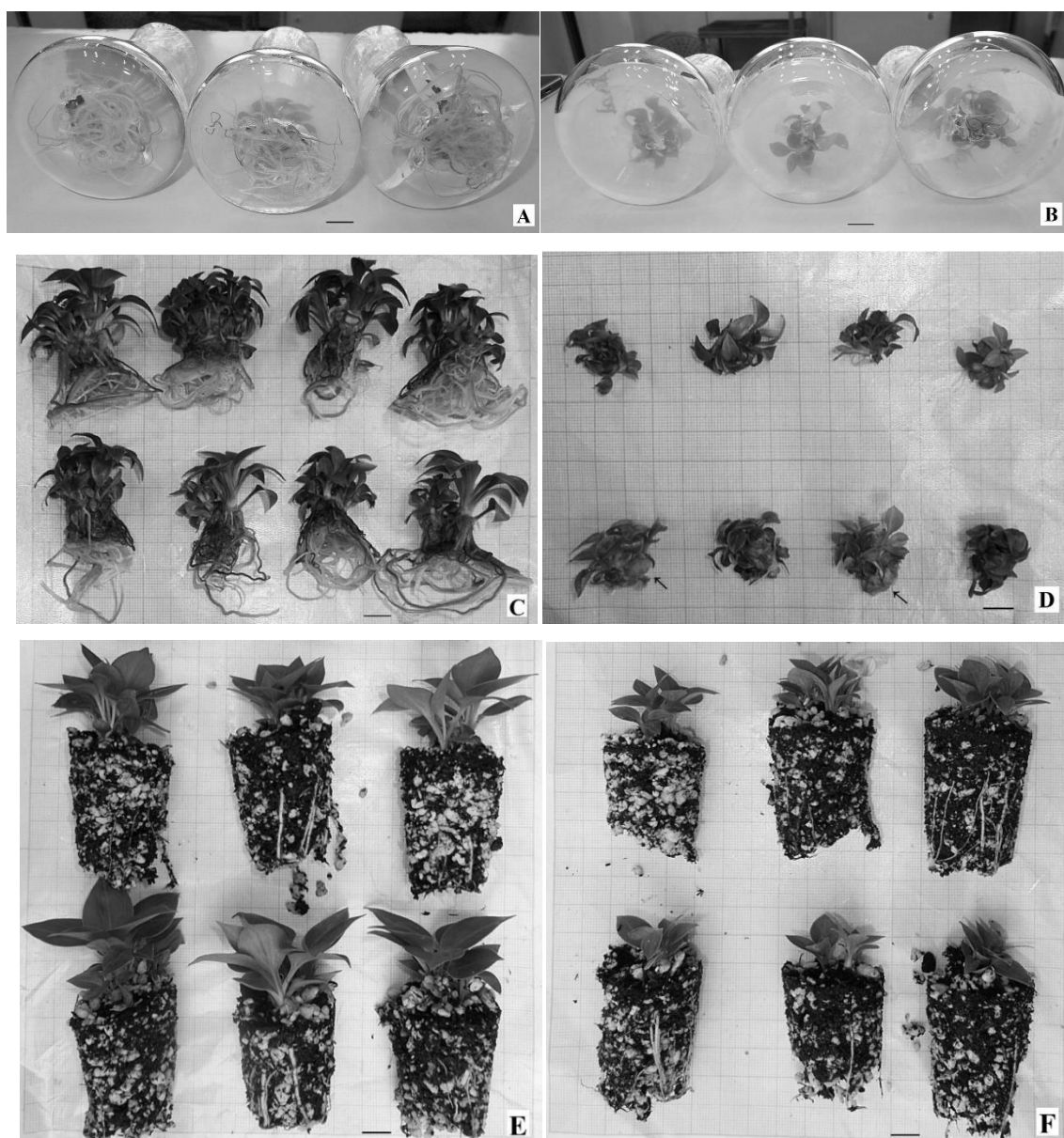


Figure 1. *Hosta* 'Gold Drop' *in vitro* and acclimatized (*ex vitro*) plants: A) Well rooted specimens in 100 ml Erlenmeyer flasks containing $\frac{1}{2}$ MS medium with 0.4 ml l^{-1} Kelpak. (B) 0.8 ml l^{-1} Pentakeep-V resulted plants without or only few, short roots. C) *In vitro* plants cultured on $\frac{1}{2}$ MS medium supplemented with 0.4 ml l^{-1} Kelpak produced more and longer shoots with larger leaves. D) Poorly developed *in vitro* plants (callus formation shown by arrows) from $\frac{1}{2}$ MS medium containing 0.8 ml l^{-1} Pentakeep-V. E) *Ex vitro* plants grown better when Kelpak was used before acclimatization. F) As after-effect, Pentakeep resulted weaker *ex vitro* plants. Scale bars on pictures: 10 mm.

Number and length of shoots

Almost every concentration of Kelpak resulted more *in vitro* shoots as compared with the control, and the highest number (5.43) were achieved on medium supplemented with 0.4 ml l^{-1} Kelpak (Figure 1 C). Similar value was detected in the case of using 0.8 ml l^{-1} Pentakeep, although this treatment resulted abnormal callus formation (Figure 1 D) as non-wanted effect in 39.28% of the *in vitro* *Hosta* 'Gold Drop' plants. Anyways, this biostimulator (especially in higher concentration) was the best for shoot multiplication of *in vitro* *Philodendron erubescens* (Asztalos, 2014).

Significantly, the longest *Hosta* 'Gold Drop' *in vitro* shoots were found when 0.4 ml l^{-1} Kelpak was added to the medium, and only this biostimulator resulted longer shoots in every concentration comparing to the control. Higher level of Pentakeep decreased length of shoots, so the shortest (16.46 mm) ones were formed with the presence of 0.8 ml l^{-1} of this agent. In contrast, *Philodendron erubescens* produced the

longest *in vitro* shoots with the use of latter biostimulator (Asztalos, 2014), and Kelpak had the worst effect in every dose if *Sorbus borbasii* 'Herkulesfürdő' was *in vitro* propagated on media contained this agent (Vidák, 2014).

The highest number of acclimatized (*ex vitro*) shoots (4.83-4.94) were found in groups of 0.2 - 0.8 ml l^{-1} Kelpak (Figure 1 E). Additionally, higher doses of Pentakeep after-effected fewer (and shorter) shoots of acclimatized plants (Figure 1 F). As compared with the other biostimulators, Kelpak was the best (as after-effect) for elongation: the longest (31.86-39.32 mm) shoots were developed in this group (Table 2). In another study (Gere, 2017), more (4.85-5.23) and longer (26.07-26.45 mm) shoots of *in vitro* *Hosta* 'Dew Drop' were found on medium containing 0.1 - 0.4 ml l^{-1} Ferbanat. As after-effect, mainly this biostimulator eventuated the greatest shooting, and higher (0.4 - 0.8 ml l^{-1}) level of Ferbanat was the best for shoot length of acclimatized plants. Therefore, biostimulators have cultivar-specific effect.

Table 2. Shoot and leaf parameters of *in vitro* and *ex vitro* *Hosta* ‘Gold Drop’ plants. Data represented by mean \pm standard deviation (SD). Means with different letter are significantly different according to Tukey’s and Games-Howell’s comparison test at $p \leq 0.05$

	Shoot number \pm SD		Shoot length (mm) \pm SD		Leaf length (mm) \pm SD		Leaf width (mm) \pm SD		
	<i>in vitro</i>	<i>ex vitro</i>	<i>in vitro</i>	<i>ex vitro</i>	<i>in vitro</i>	<i>ex vitro</i>	<i>in vitro</i>	<i>ex vitro</i>	
Control	4 \pm 2.09 ab	4.06 \pm 2.2 ab	21 \pm 2.81 bc	31.97 \pm 4.33 def	13.9 \pm 2.21 bcde	25.23 \pm 4.54 efg	6.96 \pm 1.04 abc	15.55 \pm 2.73 ef	
Ferbanat L (ml l⁻¹)	0.1	3.36 \pm 2.8 a	3.36 \pm 2.49 ab	21.86 \pm 2.54 bc	31.73 \pm 3.29 def	15.33 \pm 2.2 defg	26.13 \pm 3.72 g	7.43 \pm 1.04 bcd	15.33 \pm 2.24 ef
	0.2	2.76 \pm 1.86 a	2.7 \pm 2.02 a	21 \pm 2.75 bc	30.1 \pm 3.53 cde	14 \pm 1.91 bcdef	23.2 \pm 3.92 cdefg	7.2 \pm 0.99 abcd	13.77 \pm 2.47 cdef
	0.4	3.76 \pm 1.38 ab	4.06 \pm 2.18 ab	21.6 \pm 2.45 bc	29.07 \pm 5.87 cde	15.2 \pm 2.28 cdefg	20 \pm 4.09 bc	7.53 \pm 1.07 bcd	13.7 \pm 2.88 cde
	0.8	3.92 \pm 1.98 ab	3.46 \pm 1.73 ab	20.75 \pm 2.48 bc	25.5 \pm 4.92 b	14.64 \pm 2.32 bcdefg	17.39 \pm 4.52 ab	7.57 \pm 1.03 bcd	11.36 \pm 2.97 ab
Kelpak (ml l⁻¹)	0.1	3.17 \pm 2.05 a	3.35 \pm 1.56 ab	21.96 \pm 2.24 c	39.32 \pm 4.44 g	16.41 \pm 2.59 g	25.46 \pm 4.09 fg	7.93 \pm 0.88 d	15.46 \pm 2.82 ef
	0.2	4.3 \pm 2.57 ab	4.93 \pm 2.47 b	22.33 \pm 2.9 c	32.52 \pm 3.03 ef	16.03 \pm 3.28 fg	23.65 \pm 3.82 defg	7.83 \pm 1.14 cd	15.06 \pm 2.33 ef
	0.4	5.43 \pm 3.53 b	4.82 \pm 2.98 b	23.2 \pm 2.91 c	33.83 \pm 4.15 f	15.76 \pm 2.81 efg	24.86 \pm 5.02 efg	7.53 \pm 0.86 bcd	15.9 \pm 2.82 f
	0.8	4.2 \pm 2.32 ab	4.89 \pm 2.17 b	22.4 \pm 4.81 c	31.86 \pm 2.62 def	16.36 \pm 2.68 g	21.9 \pm 3.45 cdef	7.93 \pm 1.08 d	14.31 \pm 2.2 def
Pentakeep-V (ml l⁻¹)	0.1	4.68 \pm 3.16 ab	4.41 \pm 2.75 ab	22.48 \pm 2.74 c	30.83 \pm 2.95 cdef	13.2 \pm 1.69 bc	21.62 \pm 3.18 cde	6.96 \pm 1.01 abc	14.07 \pm 1.73 cdef
	0.2	3.89 \pm 1.81 ab	4.36 \pm 2.53 ab	21.2 \pm 2.12 bc	28.64 \pm 2.66 bcd	12.93 \pm 1.64 b	20.24 \pm 3.44 bcd	6.72 \pm 1.03 ab	12.12 \pm 1.74 bc
	0.4	3.79 \pm 2.16 ab	3.74 \pm 2.29 ab	19.41 \pm 2.84 b	28.15 \pm 3.13 bc	13.62 \pm 2.69 bcd	21.7 \pm 4.81 cde	7.06 \pm 1.3 abcd	12.41 \pm 2.17 bcd
	0.8	5.42 \pm 1.91 b	3 \pm 1.8 ab	16.46 \pm 3.2 a	22.06 \pm 2.41 a	10.17 \pm 1.92 a	15.71 \pm 1.72 a	6.28 \pm 1.15 a	9.88 \pm 1.11 a

Length and width of leaves

Significantly the longest and widest *Hosta* ‘Gold Drop’ leaves were observed on *in vitro* plants grown on medium with 0.1 and 0.8 ml l⁻¹ Kelpak (lengths: 16.41 and 16.36 mm, widths: equally 7.93 mm) comparing to the control. Similar tendency was experienced during *in vitro* propagation of *Sorbus borbasii* ‘Herkulesfürdő’ (Vidák, 2014). In our experiment, Pentakeep resulted the shortest and narrowest (*in vitro* and *ex vitro*) leaves especially when the highest dosage (0.8 ml l⁻¹) was applied (Table 2). Different treatments effected different proportions of the shoot number, - sizes and leaf parameters: if 0.8 ml l⁻¹ Pentakeep was used, there were negative correlation between the number and the length of shoots and leaves. On the other hand, Kelpak resulted more and longer shoots with larger leaves. Pentakeep, Ferbanat, Kelpak effected shorter and narrower *in vitro* *Hosta* ‘Dew Drop’ leaves as comparing to the control, but in the case of the latter 2 biostimulators’ after-effect, larger leaves were found on acclimatized plants (Gere, 2017).

Chlorophyll and carotenoid content

In vitro, there was positive coherence between chlorophyll content and size (length, width) of leaves, so the highest values were achieved in the case of 0.1 and 0.8 ml l⁻¹ Kelpak (5169.56 and 5397.33 $\mu\text{g g}^{-1}$), and the lowest (2988.33 $\mu\text{g g}^{-1}$) on medium with 0.8 ml l⁻¹ Pentakeep. Similar tendency was obtained when carotenoid contents were examined. After acclimatization, the highest chlorophyll-values (5085.76 and 5012.58 $\mu\text{g g}^{-1}$) were observed in groups previously *in vitro* propagated with the use of 0.1 ml l⁻¹ Ferbanat and 0.4 ml l⁻¹ Kelpak (and every dozens of Pentakeep after-effected the lowest levels, Figure 2). On the other hand, carotenoid contents changed differently, because significantly the highest averages (130.61-142.31 $\mu\text{g g}^{-1}$) were recorded in the case of the latter biostimulator with higher doses (Figure 3). In the study of *Hosta* ‘Dew Drop’, mainly Kelpak increased (and Pentakeep

decreased) chlorophyll and carotenoid contents of *in vitro* and acclimatized plants (Gere, 2017), however, the highest concentration of Pentakeep resulted the highest leaf pigment values of *in vitro* *Philodendron erubescens* (Asztalos, 2014), *Sorbus borbasii* ‘Herkulesfürdő’ (Vidák, 2014) and acclimatized *Phoenyx dactylifera* (Awad, 2008) plants.

Peroxidase enzyme activity

Examining *in vitro* *Hosta* ‘Gold Drop’ plants, POD activity was the lowest (0.019 U mg⁻¹) when 0.1 ml l⁻¹ Kelpak was used, and Pentakeep resulted the highest values (0.082 and 0.073 U mg⁻¹), especially when higher doses were applied (Figure 4). In Gere’s trial (2017), Pentakeep also effected the highest POD activity (0.12-0.15 U mg⁻¹) of *in vitro* *Hosta* ‘Dew Drop’, although the lowest *in vitro* values (0.03-0.07 U mg⁻¹) were detected if Ferbanat was added to the medium. In most cases, there were no significant differences between *ex vitro* POD activities, nevertheless, every concentration of Kelpak, 0.1, 0.2 ml l⁻¹ Ferbanat and 0.1, 0.4 ml l⁻¹ Pentakeep decreased (as after-effect) values as compared with control. Different results were recorded if acclimatized *Hosta* ‘Dew Drops’ were examined, Kelpak and Ferbanat was not effective for this cultivar (Gere, 2017).

Survival rate (acclimatization)

Considering total groups, 92% of plants survived acclimatization. In according to the type of biostimulator, we observed the highest rates (100%) in the case of Kelpak and Ferbanat in every concentration or 0.1 ml l⁻¹ Pentakeep, and the lowest (56%) when 0.8 ml l⁻¹ of the latter biostimulator was used. We have to mention that 100% of the control plants also successfully survived acclimatization, thus, applied biostimulators had no concrete positive effect on the number of survived specimens. Supposedly, further *ex vitro* experiments with disadvantageously modified (for example drier, colder) conditions emphasize advantages of biostimulators.

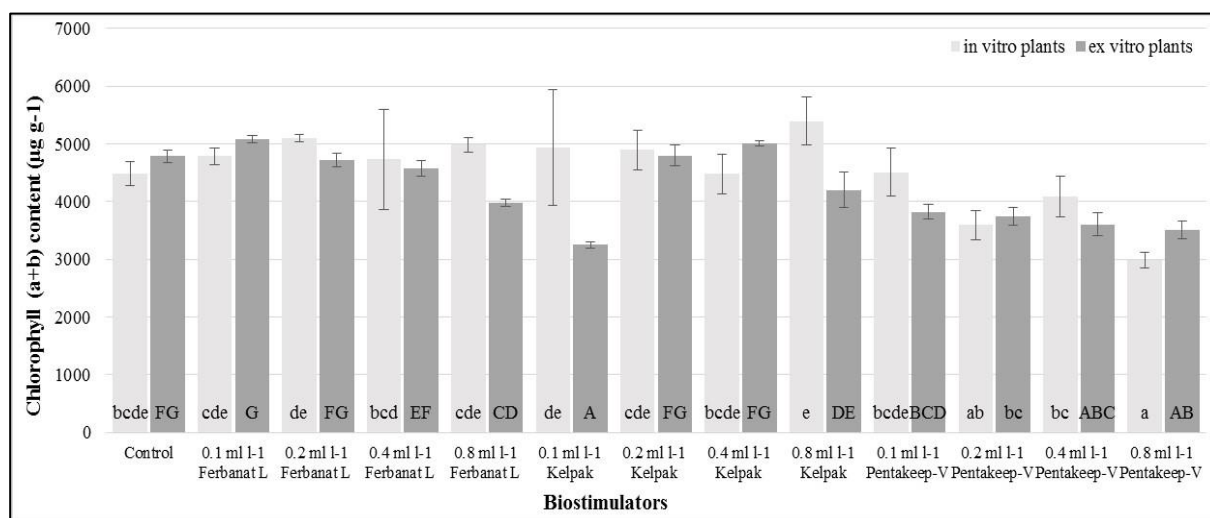


Figure 2. Chlorophyll (a+b) content of *in vitro* and *ex vitro* *Hosta* 'Gold Drop' plants. Values are mean \pm SD. Bars with different letter are significantly different by Tukey's and Games-Howell's comparison test at $p \leq 0.05$

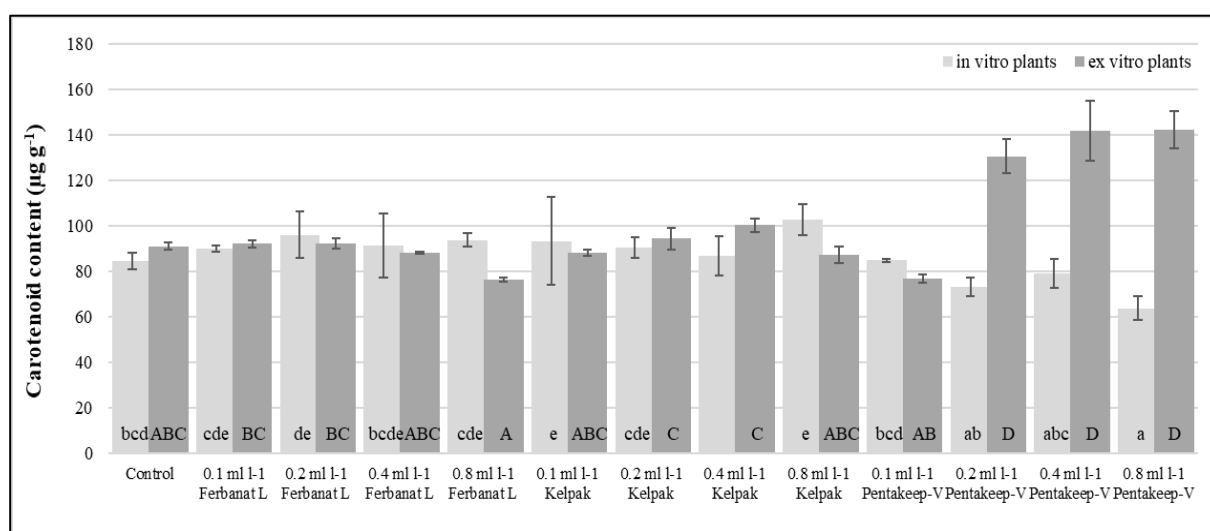


Figure 3. Carotenoid content of *in vitro* and *ex vitro* *Hosta* 'Gold Drop' plants. Values are mean \pm SD. Bars with different letter are significantly different by Tukey's and Games-Howell's comparison test at $p \leq 0.05$

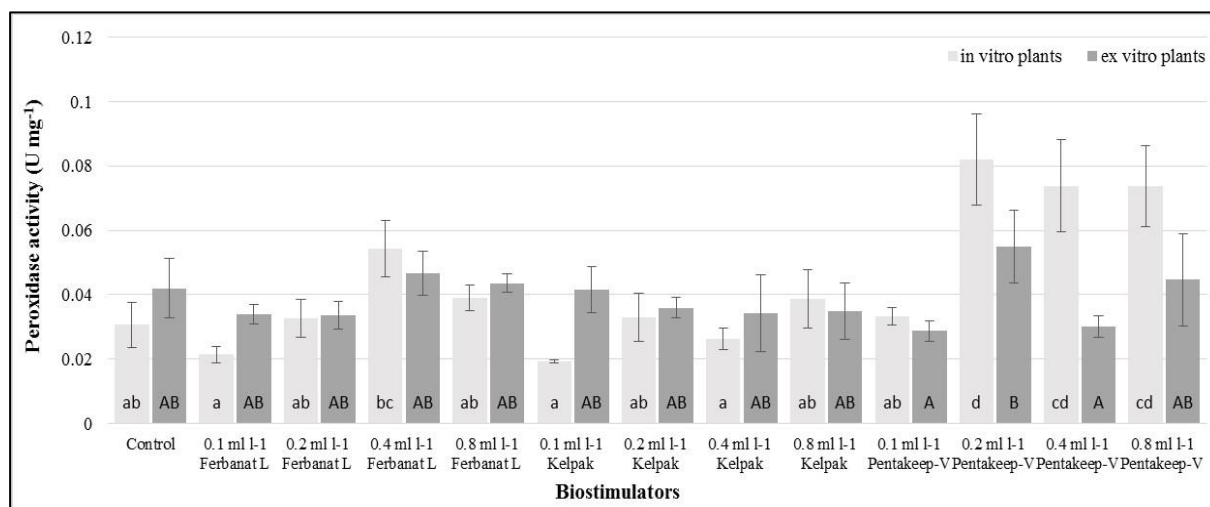


Figure 4. Peroxidase enzyme activity of *in vitro* and *ex vitro* *Hosta* 'Gold Drop' plants. Values are mean \pm SD. Bars with different letter are significantly different by Tukey's and Games-Howell's comparison test at $p \leq 0.05$

Conclusions

Summarizing, Pentakeep-V was not optimal during micropropagation of *Hosta* 'Gold Drop' because of non-wanted or bad effects. For example, this product eventuated callus development on the basal part of the shoots: almost 40% of specimens developed globular-irregular, pale green formation when medium with 0.8 ml l⁻¹ of Pentakeep was used. Root number, root length, leaf sizes (and chlorophyll, carotenoid content of leaves) also decreased in this case; therefore, almost half of these poorly grown plants did not survive acclimatization. We obtained the best results with applying Kelpak, especially in concentrations 0.2 or 0.4 ml l⁻¹. The use of this product resulted higher *in vitro* fresh plant weight definitely because of more and longer shoot with larger leaves (not only in case of *in vitro* but acclimatized plants), and probably due to the stronger habit and higher concentration of chlorophyll pigments, 100% of the shoots were successfully acclimatized. Though all control plants also survived this procedure, the use of this agent is preferred (leastwise in case of this *Hosta* cultivar) because of the higher number and larger sizes of *in* and *ex vitro* shoots. In order to ascertain positive/negative effects of these environmental friendly products, further *in vitro* and acclimatization trials with more *Hosta* taxa were suggested.

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