

# Morphological, physiological features and differences of *Vriesea splendens* 'Fire' plants during *in vitro* multiplication and rooting

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**Summary:** During *in vitro* multiplication and rooting of *Vriesea splendens* 'Fire', 0.1, 0.2, 0.4 and 0.8 mg l<sup>-1</sup> benzyladenine (BAP), benzyladenine-riboside (BAPR), kinetin (KIN), meta-topoline (MT), indole-butyric acid (IBA) and naphthalene-acetic acid (NAA) were added to basal Murashige and Skoog (1962) MS medium. As compared to the hormone-free control, plants developed significantly more shoots on medium supplemented with almost all cytokinins (excepting KIN), especially BAP resulted the highest multiplication up to almost 26 shoots. Enhancement of cytokinin concentrations increased shoot number (and in case of BAP, peroxidase activity) but decreased plant height and rooting parameters. Regarding root production, both auxins were definitely beneficial (0.2 mg l<sup>-1</sup> NAA resulted more than 7.5 roots and higher auxin concentrations efficiently stimulate root elongation); however, KIN had similar effects. After a three-month duration time of acclimatization, we observed that plants which were previously cultured on medium containing certain cytokinins (KIN in all doses and 0.1 mg l<sup>-1</sup> MT) or both auxins had greater survival, moreover, as negative after-effect, higher cytokinin concentrations reduced the number of survived specimens.

Ördögh, M. (2022): Morphological, physiological features and differences of *Vriesea splendens* 'Fire' plants during *in vitro* multiplication and rooting. International Journal of Horticultural Science 28: 78-85. <https://doi.org/10.31421/ijhs/28/2022/9656>

**Key words:** auxins, bromeliad, cytokinins, multiplication, rooting, *Vriesea*

## Introduction

Vrieseas - belong to the subfamily Tillandsioideae, with 261 species, 44 varieties (Luther, 2008) - are mostly tropical, Brazilian epiphytes with relatively small, wiry root system and high number of long, wide, often colourful (striped, spotted) leaves in rosette-shaped structure, which efficiently stores water. Because their attractive foliage and special, sword-shaped inflorescence with durable, long living red bracts and variable (yellow, white, greenish) flowers, *Vriesea* taxa (more than 150 species, hundreds of hybrids and cultivars) are very popular and important ornamental pot plants. Unfortunately, these bromeliads produce few shoots (Makara, 1982) or had low germination capability (Mercier & Kerbauy, 1995), thus, sowing or especially micropropagation give better mass propagation results, particularly in cases of higher, pathogen-free, genetically stabile, controllable shoot multiplication (Da Silva et al., 2009). Although the latter way is rather expensive and hard, several bromeliad taxa from almost all genus micropropagated successfully, such as *Ananas* (Hamad & Taha, 2008; Hamad et al., 2013; Hararap et al., 2019), *Aechmea* (Huang et al., 2010; Rosa et al., 2018; Faria et al., 2018), *Nidularium* (Jámborné et al., 2003; Paiva et al., 2009; Da Silva et al., 2012; Carvalho et al., 2013; Ördögh, 2015), *Tillandsia* (Pierik & Sprekels, 1991; Koh & Davies, 2001; Pickens et al., 2006), *Cryptanthus* (Mathews & Rao, 1982; Arrabal et al., 2002) and last but not least, *Vriesea*.

In case of *Vrieseas*, mainly endangered, endemic Brazilian species used for *in vitro* studies in order to lowering pressures on their natural, treated populations (Tamaki et al. 2011). As induction phase, mostly sterilized seeds of *V. cacuminis*

(Resende et al., 2016), *V. scalaris* (Da Silva et al., 2009); *V. gigantea* and *V. philippocoburgii* (Droste et al., 2005); *V. heliconioides* (Hernández-Meneses et al., 2018); *V. flammea* (Sasamori et al., 2020) were placed on total or half-strength Murashige & Skoog (1962) MS or Knudson (1946) K medium. Besides seeds, capsules also disinfected, but this method resulted higher contamination (Sasamori et al., 2020). In certain cases, young or basal leaves successfully used as explants in order to establish *in vitro* nodular cultures of *V. reitzii* (Alves et al., 2006; Dal Vesco & Guerra, 2010) and *V. scalaris* (Da Silva et al., 2009). In the next steps, plant growth regulators such as BAP, GA<sub>3</sub>, NAA, IBA (Resende et al., 2016; Hernández-Meneses et al., 2018), KIN (Da Silva et al., 2009), 2-iP, 2,4-D (Alves et al., 2006; Dal Vesco & Guerra, 2010) or other accessories like activated charcoal (Droste et al., 2005), ascorbic acid, hydrolysed casein, B5 vitamins (Da Silva et al., 2009) were added so as to stimulate multiplication and rooting. Not only hormones but also the effect of different concentrations of sucrose was examined during *in vitro* development and acclimatization of *V. inflata* (Freitas et al., 2015) and *V. flammea* (Sasamori et al., 2020). However, *Vrieseas* have slow growth (as usual in case of the other bromeliads, moderate development is typical), their acclimatization was not difficult, especially when *in vitro* plantlets were cultured previously on media with GA<sub>3</sub> (Resende et al., 2016; Hernández-Meneses et al., 2018) or lower values of MS macronutrients (Sasamori et al., 2020) or sucrose (Freitas et al., 2015).

The aim of our study was to find morphological and physiological differences between *V. splendens* 'Fire' *in vitro* groups and ascertain the effects of different cytokinins and auxins on the efficiency of proliferation and rooting.

## Materials and methods

### Origin of plant material

1.5-1.8 cm sized, rootless plants originated from an *in vitro* stock of *V. splendens* 'Fire', a smaller sized cultivar with 20-25 cm leaves and 30-35 cm flowers stalks (Tillyné & Honfi, 2008) were used for *in vitro* studies in the laboratory of the Department of Horticultural and Dendrology, Hungarian University of Agriculture and Life Sciences. After multiplication and rooting, the same (but larger, 3-4 cm) specimens were acclimatized in one of the greenhouse of our department.

### Culture establishment

*In vitro* plantlets were placed on Murashige & Skoog (1962) basal medium with 20 g l<sup>-1</sup> sucrose (Reanal Finomvegyészgyár Zrt., Hungary), 5 g l<sup>-1</sup> agar (Sigma-Aldrich, Merck, USA). Beside the hormone-free control, four concentrations (0.1, 0.2, 0.4 and 0.8 mg l<sup>-1</sup>, represented as "1, 2, 4 and 8" in all diagrams) of six type of plant growth regulators as benzyladenine (BAP), benzyladenine-riboside (BAPR), kinetin (KIN), meta-topoline (MT), naphthaleneacetic acid (NAA) and indole-butyric acid (IBA) were applied. The pH was adjusted 5.6 with KOH and all media autoclaved for 30 minutes on overpressure (10<sup>5</sup> Pa). Plants were illuminated by white light 40 μmol m<sup>-2</sup> s<sup>-1</sup> using 16/8 light/dark cycles and the average temperature was 20-25 °C during *in vitro* period.

Three months later, plant height and fresh weight, number of shoots, number and length of roots were examined, additionally, physiological parameters (total chlorophyll content, peroxidase enzyme activity of leaves) were also analysed. Each experiment was repeated twice and 30 plant per treatment was examined. After all measurements (as the end of our *in vitro* studies), stocks were planted in a mixture (1:1:1) of vermiculite, perlite and peat (Novobalt, Rekyva, Lithuania) and acclimatized in heated greenhouse (170 days later we calculated survival ratios).

### Measurement of physiological parameters

For determination of chlorophyll (a + b), we collect 3 x 0.1 g leaf from each medium. After preparation of leaf samples with the use of approximately 0.5 g of quartz sand + 10 ml acetone (80%) and a 24-hour long refrigeration (+4 °C) period, absorbance of suspensions was measured by GeneSys VIS-10 (Thermo Fisher Scientific Inc., USA) spectrophotometer at 644 and 663 wavelength. Leaf pigment concentration (μg g<sup>-1</sup>) were calculated by formula  $(20.2 \times A_{644} + 8.02 \times A_{663}) \times V/w$ ; where V= volume of tissue extract (10 ml), w= fresh weight of tissue (0.1 g), A= absorbance (Arnon, 1949).

Examining peroxidase (POD) enzyme activity, 3 x 0.1 g leaf from every groups were homogenized in a refrigerated mortar filled with 1.5 ml KH<sub>2</sub>PO<sub>4</sub> (pH=6.5, 0.05 M). After centrifuging (+4 °C, 20 minutes, 13500 rpm), we used extracts

without solid particles for spectrophotometric investigations (adjusted wavelength: 460 nm). For reaction, plant extracts (3 x 0.01 ml per group) were mixed with 1.7 ml C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub> (pH=4.5, 0.1 M), 0.03 ml H<sub>2</sub>O<sub>2</sub> and 0.02 ml ortodiansidine (3,3'-dimethoxybenzidine) as chromogen reagent. Enzyme activity (U mg<sup>-1</sup>) was determined with formula  $(\Delta A1 \times \text{attenuation})/\varepsilon$ ; where  $\Delta A1$  = absorbance change/1 min,  $\varepsilon$  = 11.3: extinction coefficient of ortodiansidine (Shannon et al., 1966). Three repetitions from every treatment used for examinations of all biochemical parameters.

### Data and statistical analysis

Data of shoot, root number, plant height and fresh weight, chlorophyll and peroxidase parameters were evaluated by SPSS 23.00 (IBM Corp., USA). An analysis of variance (ANOVA) was conducted to calculate the statistical significance of every data presented. In cases of detecting significant differences between treatments, recorded means were separated by Tukey's test at p < 0.05.

## Results and discussion

### Shoot number

BAP enhanced effectively the shoot multiplication, and higher (0.4 and 0.8 mg l<sup>-1</sup>) concentration resulted significantly the largest values more than 20 shoots. Additionally, higher BAP, BAPR and MT dosages also increased shoot production, thus, plants with the largest shoot-clusters were grown on the highest cytokinin levels, excepting the use of KIN. We observed the lowest averages on medium supplemented with KIN, plantlets developed usually not more than 6 shoots in these groups. During *in vitro* proliferation of *V. scalaris*, also BA was more effective than KIN, because the latter cytokinin resulted only around 1-2 shoots instead of almost 8 (da Silva et al., 2009). Also low values (chiefly between 6-8 shoots) were recorded on medium with IBA or NAA. In general, auxins rather stimulate root development than shoot proliferation, however, in case of *V. gigantea* and *V. philippocoburgii*, NAA helped formation of both shoots and roots (Droste et al., 2005). Other species such as *V. cacuminis* or *V. heliconioides* produced the best multiplication (approximately 7 or 9 shoots) when high (2.5 or 3.4 mg l<sup>-1</sup>) dose of BAP combined with 0.2 or 0.9 mg l<sup>-1</sup> NAA (Resende et al., 2016; Hernández-Meneses et al., 2018). In case of *V. reitzii*, intensified shoot development was achieved after 2 months, mainly on medium supplemented with 0.4 mg l<sup>-1</sup> NAA and 0.4 mg l<sup>-1</sup> 2-iP (Dal Vesco & Guerra, 2010). In another study, the same species' multiplication was optimal (with 60 shoots/g nodule cluster) when plants were cultured on a cytonikin mixture with BAP, KIN and 2,4-D (Alves et al., 2006).

### Plant height

Every concentration of all cytokinin increased plant height, principally, 0.4 and/or 0.8 mg l<sup>-1</sup> BAP, BAPR and MT resulted significantly shorter (mostly, 24-26.5 mm) plants if we compared other stocks cultured on hormone-free medium. On the other hand, in cases of the auxin-treated groups, we observed considerably the highest (at least 33 mm) specimens on medium containing 0.2-0.8 mg l<sup>-1</sup> NAA; thus, negative

correlation can be presumed between the number of shoots and height of plant. Hernández-Meneses et al. (2018) experienced that 0.3 mg l<sup>-1</sup> GA<sub>3</sub> efficiently elongated *V. heliconioides* stocks, which reached 76 mm after 12 weeks, but hormone-free medium also resulted higher plants around 60 mm. *V. reitzii* *in vitro* plantlets also required GA<sub>3</sub> treatment with different concentrations in order to gain similar sizes, however, the absence of this hormone similarly promoted adequate growth with at least 50 mm height after 10 weeks culturing (Dal Vesco & Guerra, 2010). Overall, it could be that the use of GA<sub>3</sub> advance better the elongation of *V. splendens* 'Fire' plants's, anyway, as Guerra and Dal Vesco reported (2010), the utilization of this plant growth regulator was necessary to develop higher *V. splendens* hybrids specimens originated from media containing thidiazuron (TDZ) which usually has cytokinin activity.

### Fresh plant weight

We found the heaviest plants on medium supplemented with NAA, most concentrations of this auxin resulted at least 1 g of weight. The lowest values (around 0.4-0.5 g) were observed in the case of KIN and MT. In addition, these plant growth regulators decreased the number of shoots and/or plant height, thus, these parameters usually correlated with their weight. The supplementation of certain accessories (such as B5 vitamins) increased shoot proliferation and fresh weight of *V. scalaris*, specimens with larger number shoots also had larger weight (Da Silva et al., 2009). In case of *V. flammaea*, higher sucrose concentrations (up to 60 g l<sup>-1</sup>) promoted larger fresh mass (Sasamori et al., 2020).

Numeric data and noticeable morphological differences were shown on **Table 1** and **Figure 1**.

### Root number and length, rooting ratio

Definitely, the highest averages (usually more than 6 roots with at least 20 mm length) were recorded in NAA-treated groups; 0.2 mg l<sup>-1</sup> NAA resulted the most, 7.58 roots and the longest ones (almost 27 mm) developed in the case of 0.8 mg l<sup>-1</sup> NAA. Furthermore, only this agent promoted 100% rooting ratio. The other hormones (including IBA) had less effect, and we obtained the lowest values especially on medium supplemented with higher dosages of BAP, BAPR or all concentration of MT (**Table 2**). In the case of a 3-month-period of *in vitro* rooting of *V. cacuminis* (Resende et al., 2016), the addition of NAA also stimulated root development more efficiently than the other auxins (IAA, IBA), the best results (2.3 roots and 14 mm length) was achieved in medium with 0.04 mg l<sup>-1</sup> NAA. Not every *Vriesea* taxa preferred auxins; the use of IBA decreased rooting of *V. scalaris* (Da Silva et al., 2009), and the highest rooting ratio (40%) was detected in the absence of this hormone, however, lower rooting connected with higher number of adventitious shoots. For *V. heliconioides*, 0.3 mg l<sup>-1</sup> GA<sub>3</sub> was optimal, averagely four roots were found on the basal parts of the *in vitro* plants (Hernández-Meneses et al., 2018). Sometimes, modifying sucrose concentration affected root parameters; for example, lower dosages (averagely 17 g l<sup>-1</sup> instead of 20-60 g l<sup>-1</sup>) enhanced *in vitro* root growth of *V. inflata* (Freitas et al., 2015), but *V. flammaea* required higher levels (usually more than 30 g l<sup>-1</sup>) for better root production (Sasamori et al., 2020). Rooting

differences shown (based on type and concentrations of auxins) on **Figure 2**.

### Total chlorophyll content

Compared with the control, most of the plant growth regulators resulted lower (in general, not higher than 1500 µg g<sup>-1</sup>) chlorophyll values; only 0.1 and 0.2 mg l<sup>-1</sup> IBA, 0.2 and 0.8 mg l<sup>-1</sup> NAA enhanced leaf pigment above 1750 µg g<sup>-1</sup>, overall, higher means were obtained on medium containing auxins (**Figure 3**). It is worth mentioning that IBA and NAA-treated plants with more (and longer) root usually had higher chlorophyll contents in their leaves. In another trial, enhancement of sucrose dose increased this parameter, during *in vitro* multiplication of *V. flammaea* (Sasamori et al., 2020).

### Peroxidase activity

Especially NAA, IBA and higher concentrations of BAP resulted significantly higher enzyme activities, around 0.2 U mg<sup>-1</sup> (**Figure 4**). Probably, certain hormones that are effectively stimulate shoot/root development or plant growth also increase physiological processes, which cause enhanced enzymatic reactions, however, unfavourable conditions, such as extreme temperatures (Duarte et al., 2019) or higher sucrose concentrations (Martins et al., 2020) can also induce stress in *Vriesea* hybrids.

### Survive the acclimatization

Most cases, plants that were previously cultured on medium supplemented with 0.1 mg l<sup>-1</sup> MT and every dosage of KIN, IBA or NAA survived better the 170-day-length acclimatization; these groups generally produced more and longer roots in higher ratios *in vitro*, and had at least 70 % survival *ex vitro*. As negative after-effect, enhancement of cytokinin (especially MT) concentration during *in vitro* multiplication decreased plant's survival (**Figure 5**). Similarly, lower concentrations (of MS macronutrients or sucrose) gave better acclimatization results of *V. flammaea* (Sasamori et al., 2020) and *V. inflata* (Freitas et al., 2015). Main phases of acclimatization were represented on **Figure 6**.

## Conclusions

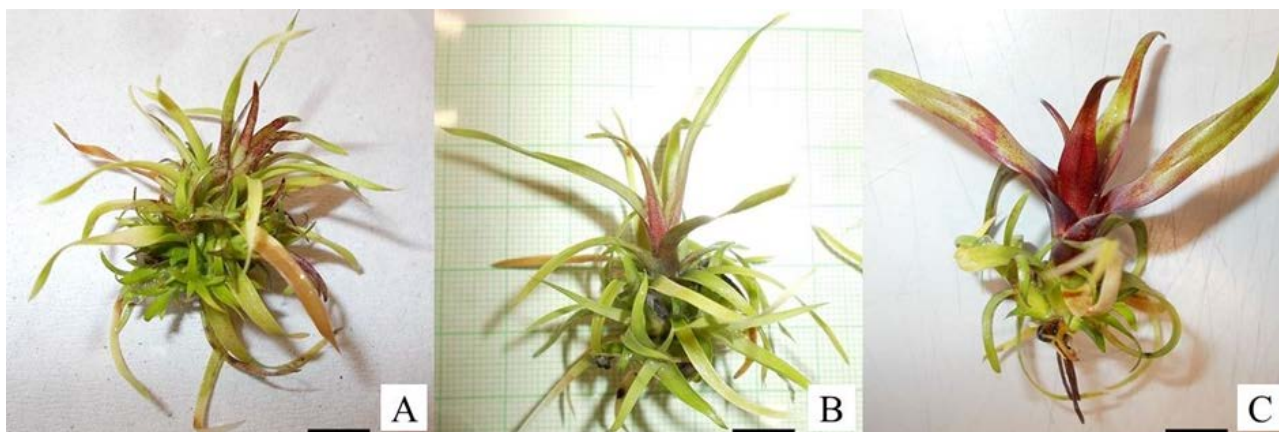
The use of BAP promoted the best proliferation and a positive relationship was found between the cytokinin concentrations and shoot number, however, higher dosages definitely decreased rooting parameters, and respectively, plants' survival chance during the acclimatization. For rooting, NAA was more suitable than IBA in every concentration; and especially 0.2 mg l<sup>-1</sup> NAA proved to be optimal, having regard to not only the trend of root development, but also the plant height and chlorophyll content. After almost half-year acclimatization period, we observed positive after-effect of both auxins and certain cytokinins (particularly KIN); these plant growth regulators previously resulted the best rooting parameters that facilitated better adaptation for *ex vitro* conditions.

**Table 1.** Root parameters of in vitro *Vriesea splendens* 'Fire' plants cultured on Murashige & Skoog (1962) basal medium containing different plant growth regulators in 0.1-0.8 mg l<sup>-1</sup> concentrations. Data represented by mean ± standard deviation (SD). Means with different letters are significantly different according to Tukey's test at p < 0.05.

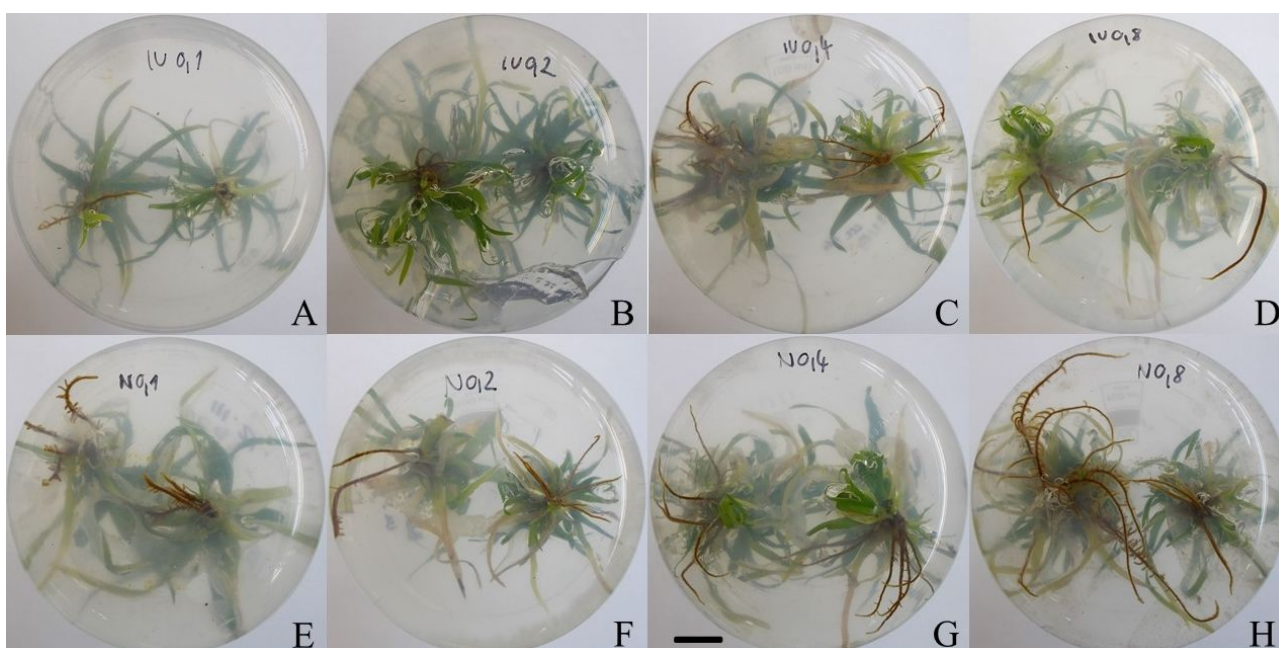
		Shoot number ± SD	Plant height (mm) ± SD	Fresh plant weight (g) ± SD
<b>Control</b>		3.84 ± 0.82 a	30.56 ± 2.56 fghi	0.48 ± 0.1 ab
<b>BAP</b> (ml l <sup>-1</sup> )	<b>0.1</b>	13.28 ± 1.24 ijk	30.17 ± 3.09 efghi	0.72 ± 0.16 cdefghi
	<b>0.2</b>	17.76 ± 3.47 l	26.78 ± 2.41 abcdef	0.86 ± 0.18 ghij
	<b>0.4</b>	21.78 ± 3.41 m	26.12 ± 2.76 abcde	0.89 ± 0.16 ij
	<b>0.8</b>	25.75 ± 3.63 n	25.65 ± 1.86 abc	0.88 ± 0.17 hij
<b>BAPR</b> (ml l <sup>-1</sup> )	<b>0.1</b>	9.96 ± 1.67 fgh	27.33 ± 3.91 abcdefg	0.86 ± 0.24 ghij
	<b>0.2</b>	12.08 ± 2.05 hij	25.77 ± 2.97 abcd	0.61 ± 0.11 abcdef
	<b>0.4</b>	15.28 ± 2.47 kl	25.13 ± 2.42 ab	0.82 ± 0.17 fghij
	<b>0.8</b>	15.88 ± 2.37 kl	25.7 ± 2.96 abcd	0.85 ± 0.17 ghij
<b>KIN</b> (ml l <sup>-1</sup> )	<b>0.1</b>	4.51 ± 1 ab	30.06 ± 4.02 defghi	0.62 ± 0.18 abcdefg
	<b>0.2</b>	6.1 ± 1 abcd	24.12 ± 3.5 ab	0.41 ± 0.1 a
	<b>0.4</b>	5.95 ± 1.18 abc	24.85 ± 2.69 ab	0.43 ± 0.09 ab
	<b>0.8</b>	5.93 ± 1.19 abc	27.68 ± 3.41 bcdefgh	0.51 ± 0.1 abc
<b>MT</b> (ml l <sup>-1</sup> )	<b>0.1</b>	9.73 ± 1.79 efgh	25.56 ± 2.52 abc	0.49 ± 0.09 ab
	<b>0.2</b>	11.26 ± 1.95 ghi	27.7 ± 2.9 bcdefgh	0.56 ± 0.09 abcde
	<b>0.4</b>	13.4 ± 2.19 ijk	23.1 ± 1.95 a	0.53 ± 0.08 abcd
	<b>0.8</b>	14.11 ± 2.09 jk	24.2 ± 2.9 ab	0.47 ± 0.11 ab
<b>IBA</b> (ml l <sup>-1</sup> )	<b>0.1</b>	3.84 ± 0.82 a	30.56 ± 2.56 fghi	0.48 ± 0.1 ab
	<b>0.2</b>	6.88 ± 1.51 bcd	31.33 ± 3.3 ghij	0.78 ± 0.23 efghi
	<b>0.4</b>	8.8 ± 2.94 defg	27.4 ± 3.13 abcdefg	0.65 ± 0.15 bcdefgh
	<b>0.8</b>	6.6 ± 2.06 abcd	32.05 ± 4.42 hij	0.78 ± 0.25 efghi
<b>NAA</b> (ml l <sup>-1</sup> )	<b>0.1</b>	6.06 ± 1.29 abcd	29.63 ± 4.04 cdefghi	0.75 ± 0.13 defghi
	<b>0.2</b>	8.25 ± 2.07 cdef	35.56 ± 4.64 j	1.03 ± 0.29 jk
	<b>0.4</b>	7.95 ± 2 cdef	33.41 ± 4.1 ij	0.93 ± 0.29 ijk
	<b>0.8</b>	7.15 ± 1.81 bcde	33.85 ± 4.13 ij	1.13 ± 0.23 k

**Table 2.** Root parameters of in vitro *Vriesea splendens* 'Fire' plants cultured on Murashige and Skoog (1962) basal medium containing different plant growth regulators in 0.1-0.8 mg l<sup>-1</sup> concentrations. Data represented by mean ± standard deviation (SD). In cases of root number and length, means with different letters are significantly different according to Tukey's test at p < 0.05.

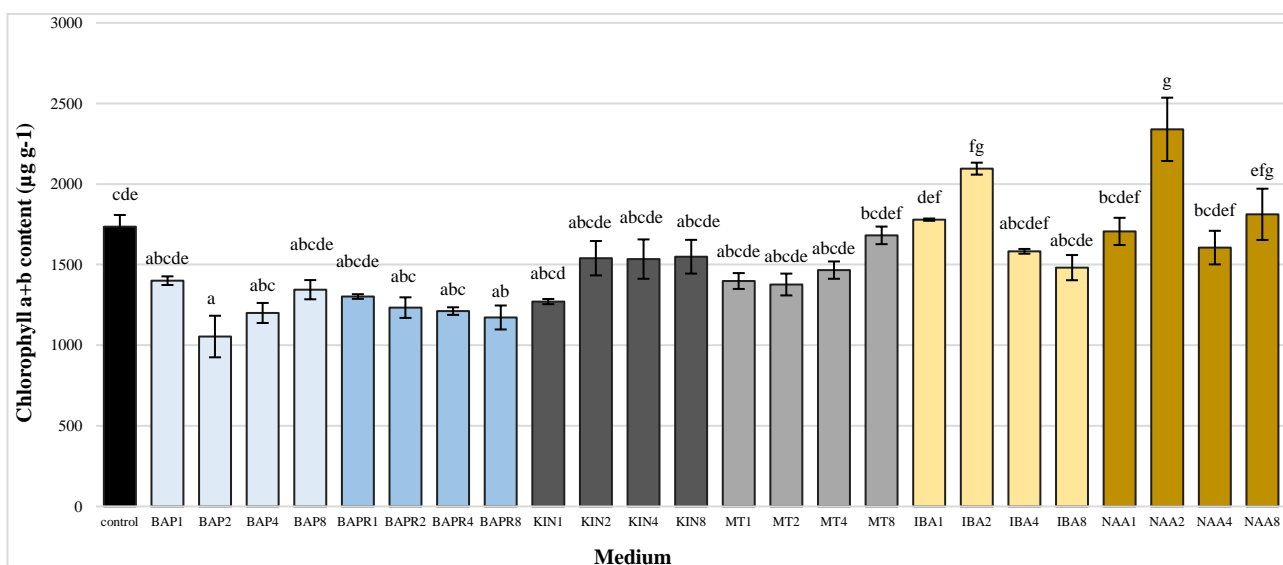
		Root number ± SD	Root length (mm) ± SD	Rooting ratio (%)
<b>Control</b>		2.24 ± 0.63 ef	9.72 ± 2.31 cde	98.3
<b>BAP</b> (ml l <sup>-1</sup> )	<b>0.1</b>	3.89 ± 0.95 g	12.01 ± 2.63 def	98.2
	<b>0.2</b>	2.41 ± 0.88 ef	7.78 ± 2.58 bc	84.8
	<b>0.4</b>	1.21 ± 0.58 abcde	3.91 ± 2.8 ab	45.6
	<b>0.8</b>	0.91 ± 6.06 abc	2.76 ± 1.8 a	46.7
<b>BAPR</b> (ml l <sup>-1</sup> )	<b>0.1</b>	1.87 ± 0.91 cdef	5.1 ± 2.38 ab	66.7
	<b>0.2</b>	1.08 ± 0.54 abcd	5.1 ± 2.67 ab	62
	<b>0.4</b>	0.95 ± 0.56abc	3.65 ± 2.18 a	51.7
	<b>0.8</b>	0.91 ± 0.53 abc	4.01 ± 2.3 ab	53.3
<b>KIN</b> (ml l <sup>-1</sup> )	<b>0.1</b>	1.81 ± 0.59 bcdef	12.7 ± 4.33 def	83.3
	<b>0.2</b>	2.24 ± 0.57 ef	9.82 ± 3 cde	89.6
	<b>0.4</b>	1.93 ± 0.6 cdef	9.83 ± 3.51 cde	88.3
	<b>0.8</b>	2.06 ± 0.55 cdef	12.96 ± 3.41 def	90
<b>MT</b> (ml l <sup>-1</sup> )	<b>0.1</b>	0.58 ± 0.45 a	2.36 ± 1.79 a	36.7
	<b>0.2</b>	0.66 ± 0.46 ab	3.33 ± 2.3 a	41.7
	<b>0.4</b>	0.43 ± 0.38 a	1.98 ± 1.85 a	28.3
	<b>0.8</b>	0.41 ± 0.35 a	2.6 ± 2.24 a	30
<b>IBA</b> (ml l <sup>-1</sup> )	<b>0.1</b>	2.24 ± 0.63 ef	9.72 ± 2.31 cde	91.4
	<b>0.2</b>	2.17 ± 0.75 def	9.27 ± 3.37 cde	82.2
	<b>0.4</b>	2.72 ± 0.62 f	14.17 ± 2.92 fg	98.4
	<b>0.8</b>	2.58 ± 0.77 f	13.5 ± 4.7 ef	87.9
<b>NAA</b> (ml l <sup>-1</sup> )	<b>0.1</b>	5 ± 1.21 g	18.21 ± 3.82 g	100
	<b>0.2</b>	7.58 ± 1.98 h	22.82 ± 3.05 h	100
	<b>0.4</b>	6.55 ± 1.71 h	23.21 ± 3.68 hi	100
	<b>0.8</b>	6.53 ± 1.01 h	26.88 ± 3.85 i	100



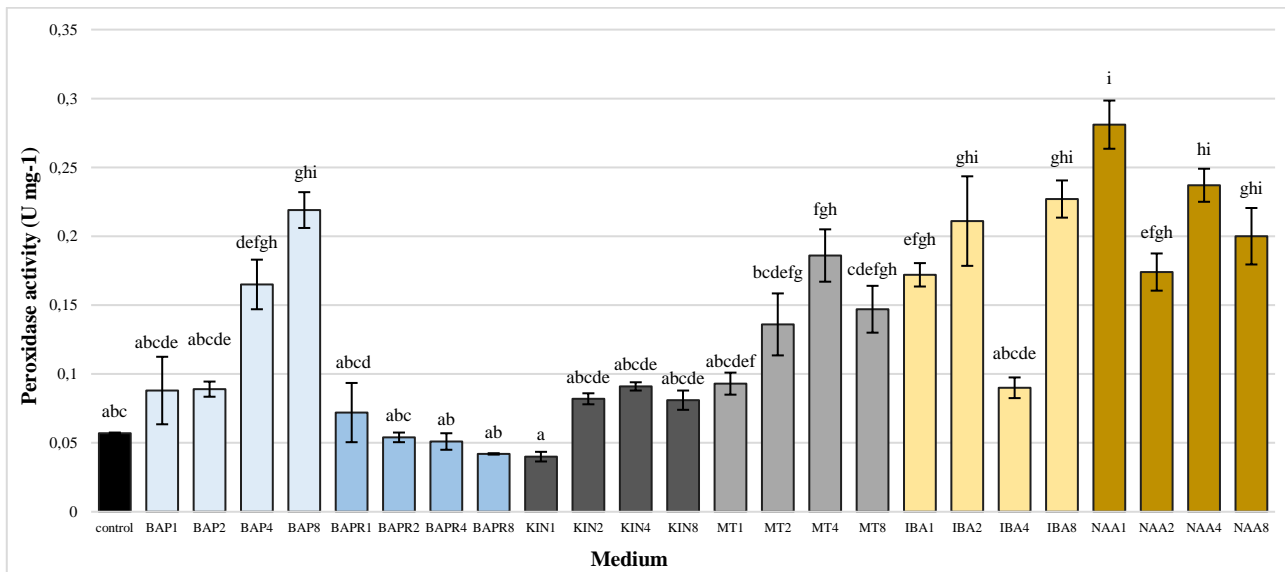
**Figure 1.** The effect of different types of hormones (in same concentration:  $0.1 \text{ mg l}^{-1}$ ) on *Vriesea splendens* 'Fire' plants' in vitro development: A) BAP resulted the highest number of shoots. B) In case of BAPR, higher plants with less shoots developed. C) NAA increased plant height, decreased shoot production and stimulate rooting. Scale bars on picture: 10 mm.



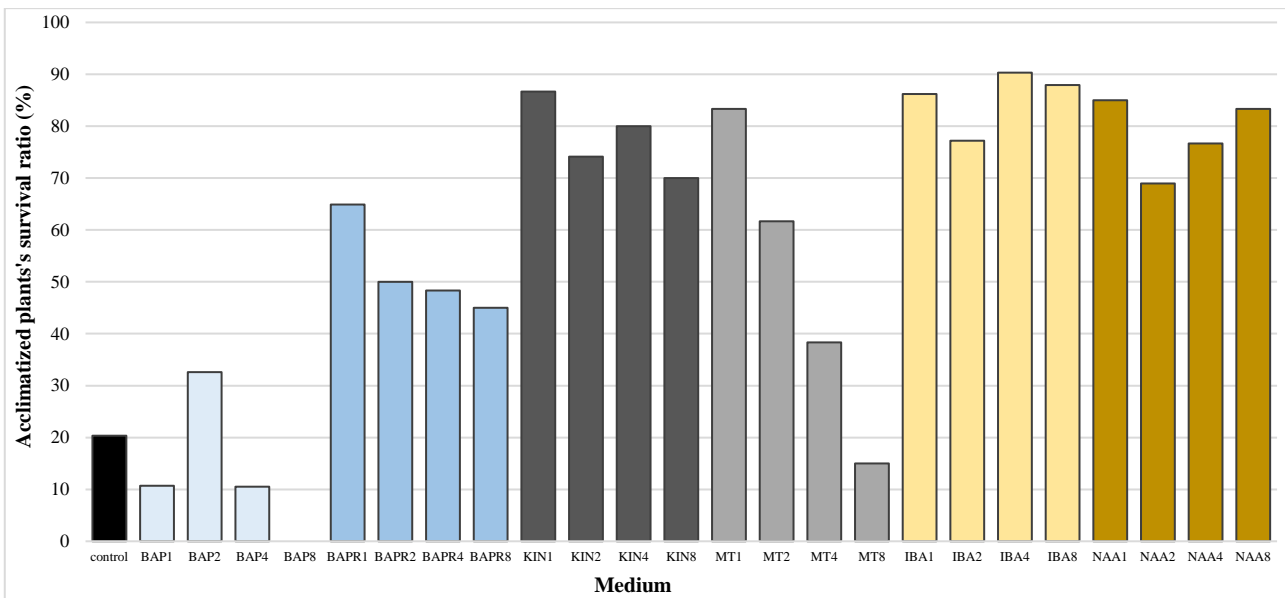
**Figure 2.** The effect of IBA and NAA on rooting characteristics of *in vitro* *Vriesea splendens* 'Fire' plants (A-B-C and D:  $0.1-0.2-0.4$  and  $0.8 \text{ mg l}^{-1}$  IBA, E-F-G and H:  $0.1-0.2-0.4$  and  $0.8 \text{ mg l}^{-1}$  NAA). Scale bar on picture: 10 mm.



**Figure 3.** Total chlorophyll (a + b) content of *in vitro* *Vriesea splendens* 'Fire' plants' leaves. Values are mean  $\pm$  SD. Bars with different letter are significantly different by Tukey's test at  $p \leq 0.05$ .



**Figure 4.** Peroxidase enzyme activity of *in vitro* *Vriesea splendens* 'Fire' plants' leaves. Values are mean  $\pm$  SD. Bars with different letter are significantly different by Tukey's test at  $p \leq 0.05$ .



**Figure 5.** Survival ratios of acclimatized *Vriesea splendens* 'Fire' plants.



**Figure 6.** Different stages of *Vriesea splendens* 'Fire' plants' acclimatization: A) Freshly cleaned *in vitro* plants before planting out into plug-tray filled with mixed substrate containing Novobalt peat, perlite and vermiculite (1:1:1). B) Three months old acclimatized plants (at time of recording their survival rate). C) 1.5 year later, survived plants were cultured in plastic pots (10 cm diameter) with the same substrate. Scale bars on picture: 10 mm.

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