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EFFECT OF ACTISIL (HYDROPLUS™), ORGANIC SUPPLEMENTS, AND PH OF THE MEDIUM ON THE MICROPROPAGATION OF *Vaccinium corymbosum*

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ABSTRACT

The effect of commercial Hydroplus™ Actisil, coconut water, coconut milk, and pH of the WPM medium on the micropropagation of *V. corymbosum* 'Liberty' was studied. Three experiments were performed with different concentrations of silicon Hydroplus™ Actisil (Si), coconut water (CW), coconut milk (CM), and different pH as a stress factor. Si was applied at a concentration of 50, 100, 200 and 500 mg dm^{-3}. The highest explant (2.02 cm) with the highest number of new shoots (1.91) and fresh weight (55.16 g) was obtained on WPM medium with the addition of Si in concentration 200 mg dm^{-3}. In experiment 2, similar to 0.1 mg dm^{-3} zeatin explant growth was achieved when 15% CW was added to the WPM medium (2.13 cm). The use of CM did not have a positive effect on blueberry growth *in vitro*. The results of experiment 3 indicated that explants of blueberry better developed when pH was lower (5.0) with the highest number of new shoots (2.85) and fresh weight (95.67g). However, there were no significant differences in plant height between pH used. The application of 200 mg dm^{-3} Actisil benefits the negative effect of higher pH of the WPM medium on micropropagation of blueberry in case of plant height, fresh weight, and biochemical parameters (proline, malondialdehyde – MDA and catalase – CAT activity).

Key words: blueberry, *in vitro*, zeatin, silicon, Woody Plant Medium, coconut water and milk

INTRODUCTION

Highbush blueberry (*Vaccinium corymbosum*) is increasingly important in fruit production due to its high content of biologically active substances, mainly flavonoids (anthocyanins, flavonols, and flavanols), procyanidins, and phenolic acids [Brazelton 2013, Schuchovski and Biasi 2019, Ochmian et al. 2020]. Rich in polyphenols, blueberry fruits show numerous health properties, improve vision, have antioxidant, anti-inflammatory, anticarcinogenic, and antibacterial effects [Schuchovski and Biasi 2019]. Recently, there has been a trend of renewing old varieties of highbush blueberry, which is closely related to global industrialization. Currently, China has become the world's fastest-growing highbush blueberry producer [Guo et al. 2019]. With the rapid expansion of blueberry cultivation scale and variety renewal, the increase in demand for healthy blueberry seedlings worldwide is inevitable. The growth of highbush blueberry bushes is limited to sites with low pH and moist soils with high organic matter content [Fin et al. 1991]. In addition, a major challenge for commercial growers is the low rooting percentage and the time required to propagate

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and market newly obtained cultivars [Schuchovski and Biasi 2019].

The use of *in vitro* cultures eliminates the limitations associated with traditional seedling propagation, providing an alternative to faster plant growth throughout the year, which increases production efficiency and profitability [Marino et al. 2014]. The best plant growth under *in vitro* conditions can be achieved by using the right composition and pH of the medium for the species and even the cultivar. Too low a pH (below 4.5) or too high a pH (above 7.0) inhibits the growth and development of *in vitro* explants [Pierik 1997]. For the initial phase of *in vitro* culture, a combination of cytokinins – mostly zeatin can usually be used [Ružić et al. 2012]. The use of natural zeatin as an additive to the culture media is expensive, therefore different solutions are being sought to give a similar effect at a lower cost. Such alternatives can be coconut water and coconut milk, which are rich sources of plant phytohormones (mainly cytokinins) [Ma et al. 2008]. Moreover, in numerous plant species, the beneficial effects of silicon on growth, development, yield, and disease resistance have been observed. Silicon is the second most ubiquitous element found in soils worldwide [Epstein 1999, Richmond and Sussman 2003, Currie and Perry 2007, Sivanesan and Park 2014]. Silicon content in soils can vary considerably from 1 to 45% of dry weight [Sommer et al. 2006], while in plants from 0.1% to 10% of dry weight [Ma and Takahashi 2002]. Hence, plants were classified into accumulating plants e.g. blueberry [Morikawa and Saigusa 2004, Figiel-Kroczyńska and Ochmian 2019], and non-accumulating plants e.g. petunia [Krupa-Małkiewicz and Calomme 2021]. Numerous authors described a positive role of Si in improving crop growth and yield under abiotic and biotic stress conditions by increasing the activity of antioxidant enzymes like CAT, increased accumulation of proline and malondialdehyde concentration (MDA), the secretion of endogenous hormones, and the production of lignins, chitinase, phenolic compounds, phytoalexin, and glucanases [Sahebi et al. 2016, Luyckx et al. 2017, Mandlik et al. 2020].

The experiment aimed in study the effect of silicon in the form of a commercial Hydroplus™ Actisil solution (Yara, Poland) and coconut water and milk on mitigating the effects of changing the pH of the medium used for the propagation of highbush blueberry. An appropriate concentration of silicon solution was selected for the micropropagation of highbush blueberry and the effectiveness of using coconut water and milk in the propagation medium as a substitute for the more expensive plant growth regulator – zeatin, was determined. Morphological (plant height, number of new shoots, fresh weight) and biochemical (proline, malondialdehyde MDA, catalase CAT activity) traits as well as leaf colour (CIE *L*a*b**) of highbush blueberry propagated on media of different composition and pH were observed.

MATERIAL AND METHODS

Characteristics of the area of research and plant material. Three separate experiments were conducted in the laboratory of the Department of Plant Genetics, Breeding and Biotechnology at the West Pomeranian University of Technology in Szczecin, Poland in 2020.

Silicon. In the first stage, the experiment on selection of silicon solution concentration with the most favourable effect on the morphological characters of the tested blueberry plants was performed. The plant material consisted of 17–20 mm shoots of *V. corymbosum* 'Liberty' obtained from sterile stabilized *in vitro* culture cultivated on the McCown Woody Plant medium – WPM [Lloyd and McCown 1981, Duchefa Biochemie B.V, The Netherlands]. The shoot explants were transferred to WPM medium with the addition of commercial silicon solution (Hydroplus™ Actisil, Yara Poland) in concentrations of 50, 100, 200, and 500 mg dm^{-3}. The silicon (0.6% Si) contained in Hydroplus™ Actisil was in the form of orthosilicic acid (H_4SiO_4), stabilized with choline. The pH of all media was adjusted to 5.8.

Coconut water (CW) and coconut milk (CM). The second experiment was conducted to establish the effects of the optimal concentration and source of promoting growth substance (coconut water and coconut milk) on the morphological growth parameters of *V. corymbosum* 'Liberty' as a cheaper substitute for plant growth hormone – zeatin. Explants (17–20 mm) were multiplied on the WPM medium supplemented with CW and CM in the concentration of 10% and 15% both. The control was WPM medium with the addition of 0.1 mg dm^{-3} zeatin. The pH of the medium

was adjusted to 5.8. Both coconut milk and water were added to the WPM medium before autoclaving.

Based on the two initial experiments, the concentration of Hydroplus™ Actisil equal to 200 mg dm^{-3} and coconut water 15% which gave the most beneficial effects on the morphological characteristics of the *V. corymbosum* 'Liberty' explants were used in the third (target) experiment.

pH, silicon, coconut water. The third experiment was conducted to establish the effect of commercial silicon solution and coconut water in alleviating the negative influences of pH on *V. corymbosum* growth and development. The explants (17–20 mm) were transferred to the WPM medium with different pH (5.0, 5.5, and 6.0) with or without 200 mg dm^{-3} Hydroplus™ Actisil, Yara or 15% coconut water.

General culture conditions. All media were supplemented with 3% (w/v) sucrose (Chempur, Poland), 0.8% (w/v) agar (Biocorp, Poland), 100 mg dm^{-3} myo-inositol (Duchefa Biochemie B.V, The Netherlands), heated and 30 ml were poured into a 450 ml flask. Next, they were autoclaved at 121°C (0.1 MPa) during the time required according to the volume of medium in the vessel. Cultures were incubated in a growth room at a temperature of 24 ±2°C under 16 hours photoperiod with a photosynthetic photon flux density (PPFD) of 40 µmol $m^{-2}s^{-1}$ provided by Narva (Germany) emitting daylight cool white. Each combination included 32 shoots (8 replications with 4 explants per flask). After 35 days, explants were removed and wash with deionized distilled water, and shoot and root length (cm), number of new shoots, and determination of colour were measured. The explants were weighed for estimation of plant fresh mass (g).

Determination of colour. The pigment (colour) of leaves (from the middle part of the shoot) was measured in transmission mode by photocolorimetric method in CIE *L*a*b** system [Hunterlab 2012] using spectrophotometr CM-700d (Konica Minolta, Japan). The diameter of the measurement hole was 3 mm, the observer type 10°, and the illuminant D65. The value of *a** was range from green (–*a**) to red (+*a**). The parameter *b** described the colour in the range from yellow (+*b**) to blue (–*b**). The value of parameter *L** means monochromaticity in the range from 0 (black) to 100 (white).

Analysis of proline, malondialdehyde (MDA), and determination of catalase (CAT) activity (EC 1.11.1.6). The concentration of proline was determined according to the method of Bates et al. [1973] using spectrophotometer at 520 nm in blueberry fresh leaves and calculated as µmol g^{-1} FW. The content of the malondialdehyde (MDA, a product of lipid peroxidation) in plant tissue was determined by the method described by Sudhakar et al. [2001]. The concentration of MDA was calculated from the absorbance at 600, 532, and 450 nm, and MDA contents were estimated using the following equations:

$$\text{MDA (nmol g}^{-1}\text{ FW)} =$$
$$= [6.45 \times (A532 - A600) - 0.56\ A450] \times V/FW,$$

where: V – volume of the sample, A – absorbance, FW – fresh weight.

The catalase (CAT) activity was determined according to the method by Lück [1963]. The decrease in absorbance, caused by the decomposition of H_2O_2, was monitored continuously at 240 nm for 90 s. One unit of enzyme is the amount necessary to decompose 1 µM H_2O_2 g^{-1} FW min^{-2}.

Statistical analysis. All statistical analyses were performed using Statistica 13.0 (StatSoft Polska, Cracow, Poland).

Statistical significance of the differences between means was determined by testing the homogeneity of variance and normality of distribution, followed by ANOVA with Tukey's post hoc test. The significance was set at $p < 0.05$. The relationship between morphological (plant height, number of new shoots, and fresh weight) and biochemical (proline, MDA, CAT activity) traits of highbush blueberry grown under *in vitro* conditions was illustrated.

RESULTS AND DISCUSSION

Application of Hydroplus™ Actisil in the WPM medium

Plant tissue culture media contain both non-organic and organic nutrients that support healthy plant growth. To improve growth and morphogenesis *in vitro*, the culture medium is often optimized and the extent of modification depends largely on the species

or even the cultivar [Sahebi et al. 2015]. The effect of Si in the nutrient solution was studied by several authors. Rodrigues et al. [2017] describe that silicon as a sodium silicate at a concentration of 1–2 mg dm^{-3} promotes better yam (*Dioscorea* spp.) development *in vitro*. Gallegos-Cedill et al. [2018] showed that the inclusion of Si (Siliforte®) to the nutrient solution of *V. corymbosum* L. cv. Ventura benefits from its vegetative growth. However, Costa et al. [2020] have used potassium silicate (K_2SiO_3) at a concentration of 1 mg mg dm^{-3} in liquid MS medium to propagate 'Dwarf Cavendish' banana in bioreactors. Their study demonstrated that silicon solution improved plantlet growth. However, the mechanism of the supplementation effect of Si on plant height is unclear [Kamenidou et al. 2008]. According to Morikawa and Saigusa [2004] Si is not known to be an essential element for the growth of blueberries, rather it was the element that accumulated mostly in the leaves. In addition, Si is not included in any commercial basal medium for plant tissue culture, may only be present in trace amounts.

In this study, the effect of commercial silicon solution (Hydroplus™ Actisil) supplementation on morphological traits (plant height, number of new shoots, fresh weight) and colour of the blueberries leaves propagated *in vitro* depended on its concentrations (Tab. 1, Fig. 1). It was observed that concentrations of 50, 100, and 500 mg dm^{-3} statistically decrease plant height from 50 to 41%, in comparison to the concentration of 200 mg dm^{-3} (2.02 cm). Also, the number

Table 1. Effect of different concentrations of silicon (Hydroplus™ Actisil) in the WPM medium on morphological traits and leaves colour (using CIE *L*a*b** system) of *V. corymbosum* 'Liberty' under *in vitro* condition comparison to the WPM medium with 0.1 mg L^{-1} zeatin, after 53 days of culture (n = 32 shoots per treatment)

		WPM medium				
		Zeatin (mg dm^{-3})	Hydroplus™ Actisil (mg dm^{-3})			
		0.1	50	100	200	500
Color parameters CIE leaves	*L**	36.40c*	29.25a	35.04bc	35.45c	31.62ab
	*a**	10.55a	10.22a	10.25a	12.64a	10.26a
	*b**	19.66a	15.76a	16.18a	21.00b	15.20a
Plant height (cm)		2.15b	1.19a	1.02a	2.02b	1.02a
Number of new shoots		2.75c	1.41a	1.38a	1.91b	1.13a
Fresh weight (mg)		36.36a	37.47a	50.61ab	55.16b	38.83a

*Means followed by different letters in columns are significantly different at 5% level according to Tukey's multiple range

WPM + 0.1 mg dm^{-3} zeatin, WPM + 50 mg dm^{-3} Actisil, WPM + 100 mg dm^{-3} Actisil, WPM + 200 mg dm^{-3} Actisil, WPM + 500 mg dm^{-3} Actisil

Fig. 1. *In vitro* grown *V. corymbosum* 'Liberty' plantlets after 35 days of culture on WPM medium with the addition of 0.1 mg dm^{-3} zeatin and different concentrations of Hydroplus™ Actisil

of new shoots per plant and plant fresh weight statistically decreased (from 41 to 26% and from 32 to 8%, respectively) when the concentration of 50, 100, and 500 mg dm^{-3} was used in WPM medium (Tab. 1).

Our results and the reported literature [Sivanesan and Park 2014, Rodrigues et al. 2017, Costa et al. 2020] indicated the positive role of silicon on the growth and development of several species, including an increase in the number of leaves and fresh weight of plants grown *in vitro*. The positive effect of Si on plants growth may be due to increased nutrient uptake and photosynthetic activity.

In our study, it was confirmed that according to the producer recommendation the commercial silicon solution (Hydroplus™ Actisil) at a concentration of 200 mg dm^{-3} is optimal for blueberry cv. Liberty *in vitro* cultivation.

The obtained results of the colour determination of the leaves analyzed in the transmitted mode using the photocolorimetric method in the CIE *L*a*b** system [Krupa-Małkiewicz et al. 2019], showed that the addition of 200 mg dm^{-3} Hydroplus™ Actisil to the WPM medium affected the parameter *L** of the highbush blueberry leaves as compared to the three other concentrations used in this study (Tab. 1). The value of which ranged from 29.25 (50 mg dm^{-3} Actisil) to 35.45 (200 mg dm^{-3} Actisil). It was observed that silicon solution did not affect the intensity of the green colour (parameter *a**). There were no significant differences among the examined four groups of plants. However, the colour of highbush blueberry leaves with an addition 200 mg dm^{-3} Hydroplus™ Actisil, was from 23 to 28% more yellow than the other three groups (Tab. 1).

Application of coconut water (CW) and coconut milk (CM) in the WPM medium

Coconut water and coconut milk were used to enhance axillary shoot growth compared to the more expensive zeatin, to precondition potential explants that were later used as a source of shoot explants in a further experiment. To stimulate *in vitro* responses, CW is usually added to the medium at concentration ranging from 5 to 30%. However, the optimum concentration varies among plant species and must be empirically determined [Al-Khayri 2010]. A number of authors suggested that the addition of 10% or 15% CW improved the number of shoots per explant, promoted callus induction and somatic embryogenesis, for instance in *V. corymbosum* [Fira et al. 2008], leaftea *Melothria maderaspatana* [Baskaran et al. 2009], lemon grass *Cymbopogon pendulus* [Bhattacharya et al. 2010]. Based on empirical experimentation involving a range of concentrations of CW, these studies have determined the optimum concentration of CW which would allow a similar effect to that of 0.1 mg dm^{-3} zeatin and would be a less expensive alternative.

The addition of 0.1 mg dm^{-3} zeatin or 10% and 15% CW to the WPM medium had a statistically significant effect on subsequent growth, as shown by the increased mean values for all shoot parameters in comparison to the CM (Tab. 2, Fig. 2). The plant height and number of shoots grown on WPM medium with 15% CW were similar compared to the WPM medium with the addition of 0.1 mg dm^{-3} zeatin. Whereas, the addition of CM to the WPM medium inhibited growth and shoot development of blueberry. On this medium plants grow slowly and no new shoots per plant were developed. Also, fresh weight (FW) in this group was from 49% to 54% lower than for plants grown on medium containing 0.1 mg dm^{-3} zeatin. The use of CM in the WPM medium did not have a positive effect on blueberry growth *in vitro*. The reason for this may have been that the high fat content of coconut milk can have a damaging effect on cell structure [Harkacz et al. 1997]. The fat may have disrupted osmosis and flow in the micropropagated blueberries explant. Therefore, coconut water was chosen for later studies.

In this study, it was observed that the addition of coconut water and zeatin to the WPM medium affected the colour of the highbush blueberry leaves as compared to the medium with coconut milk (Tab. 2). The leaves of plants on medium with CW and zeatin had the significantly brightest color and it was evidenced by parameter *L**. It was observed that the addition of CM and CW to the WPM medium, regardless of concentration, had no significant influence on the intensity of the green colour (parameter *a**). Measured on the blueberry leaf surface, that valus ranged from –1.83 (15% CM) to 1.34 (10% CW), in comparison to the 0.1 mg dm^{-3} zeatin (–6.55). However, it was observed that addition to the WPM medium CM significantly reduced the parameter *b** (indicated the location along the axis between yellow and blue). Blueberry leaves

Table 2. Effect of different concentrations of coconut water and coconut milk in the WPM medium on morphological traits and leaves colour (using CIE *L*a*b** system) of *V. corymbosum* 'Liberty' under *in vitro* condition comparison to the WPM medium with 0.1 mg L^{-1} zeatin, after 53 days of culture (n = 32 shoots per treatment)

		WPM medium				
		0.1 mg dm^{-3} zeatin	coconut water 10%	coconut water 15%	coconut milk 10%	coconut milk 15%
Color parameters CIE leaves	*L**	46.40c	42.36bc*	46.71c	34.08a	34.80ab
	*a**	–6.55b	2.12a	1.18a	2.21a	2.28a
	*b**	29.66c	26.33c	24.82c	20.03a	20.88ab
Plant height (cm)		2.15b	1.98b	2.13b	1.27a	1.29a
Number of new shoots		2.75b	1.13a	2.52b	1.00a	1.00a
Fresh weight (mg)		36.36b	31.33b	30.13b	16.96a	19.00a

*Means followed by different letters in columns are significantly different at 5% level according to Tukey's multiple range

WPM + 0.1 mg dm^{-3} zeatin WPM + 10% CM WPM + 15% CM WPM + 10% CW WPM + 15% CW

Fig. 2. *In vitro* grown *V. corymbosum* 'Liberty' plantlets after 35 days of culture on WPM medium with the addition of 0.1 mg dm^{-3} zeatin and different concentrations of coconut milk (CM) and coconut water (CW)

growing in the WPM medium with the addition of zeatin or CW (independence of its concentrations) indicated a higher value of parameter *b** (25.80–29.66) as compared to the other explants (Tab. 2).

Many authors [Peixe et al. 2007, Yong et al. 2009, Al-Khayri 2010] reported that coconut water contains mainly water (94%) and growth-promoting substances that influence *in vitro* cultures including inorganic ions, amino acids, nitrogenous compounds. CW and CM also appear to have growth regulatory properties, e.g., auxin, various cytokinins, GAs and ABA which are a class of phytohormones [Boase et al. 1993, Ma et al. 2008, Yong et al. 2009]. According to Yong et al. [2009] the cytokinin found in coconut water support cell division, and thus promote rapid growth. Other than in our study results described Boase et al. [1993], who indicated that the addition to the BAP-based MS medium 10% of coconut milk can improve shoot responses of *in vitro* grown kiwifruit, both males and females. As Peixe et al. [2007] and Al-Khayri [2010] points out, the use of natural organic compounds to replace expensive chemical sources of plant growth regulators can reduce the cost of micropropagation and consequently lower the market price for seedlings.

Effect of variable pH, silicon, and coconut water on in vitro development and growth of highbush blueberry

The effect of stress on plant growth is often determined by measuring shoot and root length [Krupa-Małkiewicz et al. 2019]. Low pH (high H^+ activity) can directly inhibit plant growth and development

[Kidd and Proctor 2001, Pavlovkin et al. 2009], possibly through adverse effects at the root plasma level, as well as through reduced uptake and translocation of mineral nutrients [Martins et al. 2011]. One of the limiting factors for the development and production of blueberry is the pH of the soil. It is well known that blueberries develop well in acidic soil with a pH of 4.5 to 5.5 and with low levels of fertility [Gallegos-Cedillo et al. 2018]. According to Fira et al. [2008] the pH recommended for the media used for Blue Crop *in vitro* propagation is 5.

When the blueberry 'Liberty' explants were cultured on media with different pH levels and with the addition of Hydroplus™ Actisil or coconut water, showed significant differences in plant height, a number of new shoots, FW, and biochemical traits tested (proline, MDA, and CAT activity) (Tab. 3 and 4, Fig. 3). In cultivar Liberty the mean plant height was 1.90 cm, regardless of the pH of the medium. The addition to the WPM medium 200 mg dm^{-3} of Hydroplus™ Actisil has increased in the plant height of 10%, and CW caused a reduction in shoot growth of 7%, in comparison to the control. Similarly for the mean FW, Si solution caused an increased of 26% of mean FW, but CW – decreased of 48% of mean FW compared to the control (74.1 mg). In contrast, both Actisil and CW reduced the number of new shoots per plant by 40% and 28%, respectively (Tab. 3). While no significant differences were observed for different pH of the WPM medium for such traits as mean plant height and mean number of shoots per plant, agreed with the visual observations that plants grown at higher pH were about the same height but had smaller leaves and were less vigorous when compared with the plants at low pH (Tab. 3). Although the mean plant FW was significantly lower on pH 6 of WPM medium compared to the other pH used. Similar results were obtained by Finn et al. [1991] when the tolerance of higher pH in the seedlings of *V. angustifolium* and *V. corymbosum in vitro* was studied. They have proposed that *V. augustifolium* could be a source of genes for tolerance to high pH. In other hand, Tsuda et al. [2014] showed a varied response of highbush blueberry to different pH levels (pH from 4 to 7) of culture medium. Moreover, they indicated that *in vitro* screening method could become a very useful tool for the selection of germplasm with tolerance to higher pH with very small planting within a short time.

Table 3. Effect of 200 mg dm^{-3} Hydroplus™ Actisil and 15% coconut water (CW) in the WPM medium with different pH on morphological traits of *V. corymbosum* 'Liberty' under *in vitro* condition after 53 days of culture (n = 32 shoots per treatment)

		WPM medium			
	pH	control	200 mg dm^{-3} Actisil	15% CW	mean
Plant height (cm)	5.0	1.93b*	2.07c	1.91b	1.97A
	5.5	1.86b	2.33d	1.51a	1.90A
	6.0	1.91b	1.83b	1.88b	1.87A
Mean		1.90B	2.08C	1.77A	
Number of new shoots	5.0	2.9f	1.3a	1.9cd	2.0A
	5.5	2.2de	1.7bc	1.8bc	1.9A
	6.0	2.5e	1.6b	1.8bc	2.0A
Mean		2.5C	1.5A	1.8B	
FW plant (mg)	5.0	95.7d	75.2c	40.0a	70.3B
	5.5	71.4c	123.4e	34.6a	76.5B
	6.0	55.1b	81.3c	40.9a	59.1A
Mean		74.1B	93.3C	38.5A	

*Means followed by different letters in columns are significantly different at 5% level according to Tukey's multiple range

Table 4. Effect of 200 mg dm^{-3} Hydroplus™ Actisil and 15% coconut water (CW) in the WPM medium with different pH on biochemical traits of *V. corymbosum* 'Liberty' under *in vitro* condition after 53 days of culture (n = 32 shoots per treatment)

		WPM medium			
	pH	control	200 mg dm^{-3} Actisil	15% CW	mean
Proline (μmol g^{-1})	5.0	15.3b*	12.2a	27.8e	19.6A
	5.5	19.8cd	15.7b	33.1f	21.7AB
	6.0	22.4d	17.8bc	31.7e	24.0B
Mean		19.2B	15.2A	30.9C	
MDA (nmol g^{-1})	5.0	18.8a	22.3b	43.0f	28.0A
	5.5	26.0c	17.5a	39.3e	27.6A
	6.0	35.5d	19.8ab	37.8de	31.0A
Mean		26.8B	19.9A	40.0C	
Catalze (μM H_2O_2 g^{-1} min^{-2})	5.0	61.1a	70.4bcd	76.8de	69.4A
	5.5	69.0bc	63.8ab	87.3f	73.4A
	6.0	72.3cd	67.9abc	83.2ef	74.5A
Mean		67.5A	67.4A	82.4B	

*Means followed by different letters in columns are significantly different at 5% level according to Tukey's multiple range

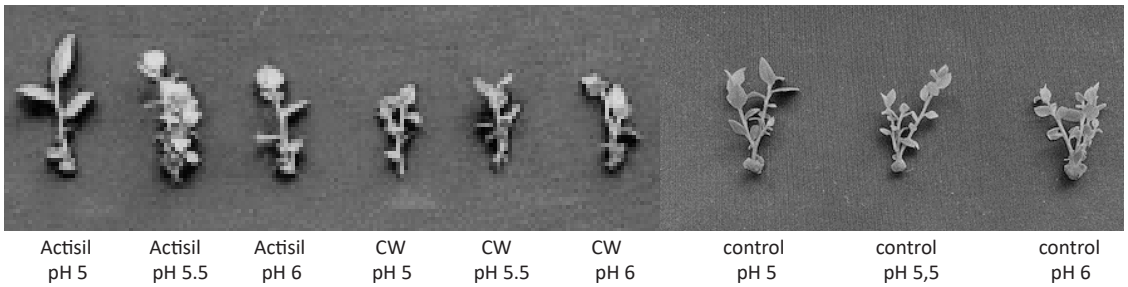

Fig. 3. *In vitro* grown *V. corymbosum* 'Liberty' plantlets after 35 days of culture on WPM medium with/without the addition of 200 mg dm^{-3} Hydroplus™ Actisil (Si) or 15% coconut water (CW) and different pH (5.0; 5.5; 6.0) as a control

Krupa-Małkiewicz and Calomme [2021] investigated the effects of different concentrations of Hydroplus™ Actisil on the morphological and biochemical traits of petunia under *in vitro* and greenhouse conditions. It was found that the addition of commercial silicon solution to the MS medium decreased plant height and root length but increased proline, MDA, total polyphenol and CAT activity, which may indicate that silicon provokes a stress response of the *in vitro* plants. In contrast, Gallegos-Cedillo et al. [2018] indicated that application of 1.2 mM of silicon (Siliforte®) benefits significantly the vegetative growth of blueberry plants (*V. corymbosum* L. cv. Ventura). They observed that increase the pH level to 6.25 decreased the dry weight of stem and leaves of blueberry. Maximum vegetative development was obtained at pH values ranging from 4.00 to 5.50 in coir fiber. In other hand, Fira et al. [2008] confirmed that it is possible to use CW to the medium instead of zeatin but a low number of new shoots were obtained and the explants grow slowly.

Biochemical parameters of the stress response

According to many authors [Barbosa et al. 2015, Krupa-Małkiewicz and Smolik 2019] elevated proline and MDA levels in plant tissues are quite a good indicator of the negative effects of various stress factors on a plant. Under the influence of stress factors, the production and accumulation of proline occurs, which provides protection to the cells by lowering the osmotic potential and drives water uptake. Many studies demonstrate that plants tolerant to different environmental stress factors accumulate more proline, than sensitive plants. An increased catalase activity may also indicate that a stress factor is acting on the plant. According to Sędzik et al. [2019], CAT is the major antioxidant enzyme associated with scavenging oxygen species (ROS).

In the present study, it was observed that as the pH of the medium increased, stress levels in plants increase, as indicated by increasing concentrations of proline, MDA and catalase activity (Tab. 4). The addition of Hydroplus™ Actisil to the WPM medium increased the concentrations of the tested biochemical parameters only when pH 5.0 of the medium was used, and decreased when the pH of the medium was 5.5 or 6.0, compared to the control. Deactivation of CAT is linked with decreasing concentration of the MDA, suggesting that oxidative damage may be reduced by the addition of Actisil. In contrast, proline concentration increased from 42 to 82% compared to the control, when the addition of 15% coconut water was used. Similarly, the addition of 15% CW to the WPM, regardless of the pH, increased concentrations of MDA (from 6 to 129%) and CAT activity (from 16 to 27%), compared to the control. The results of this study are in line with previous findings which showed Si as a plant-beneficial element associated with the mitigation of abiotic stresses [Barbosa et al. 2015, Gallegos-Cedillo et al. 2018].

Leaf colour

Many authors [Piwowarczyk et al. 2016, Krupa-Małkiewicz and Calomme 2021] suggested that environmental stress factors have a negative effect on the photosynthetic pigment content. According to Krupa-Małkiewicz and Calomme [2021] contents of photosynthetic pigment in leaves are closely correlated to their colour. In this study, it was observed that leaves of highbush blueberry differed significantly in colour (Fig. 4). The value of parameter *L** (reaching from 0 to 100, black and white, respectively) is usually used

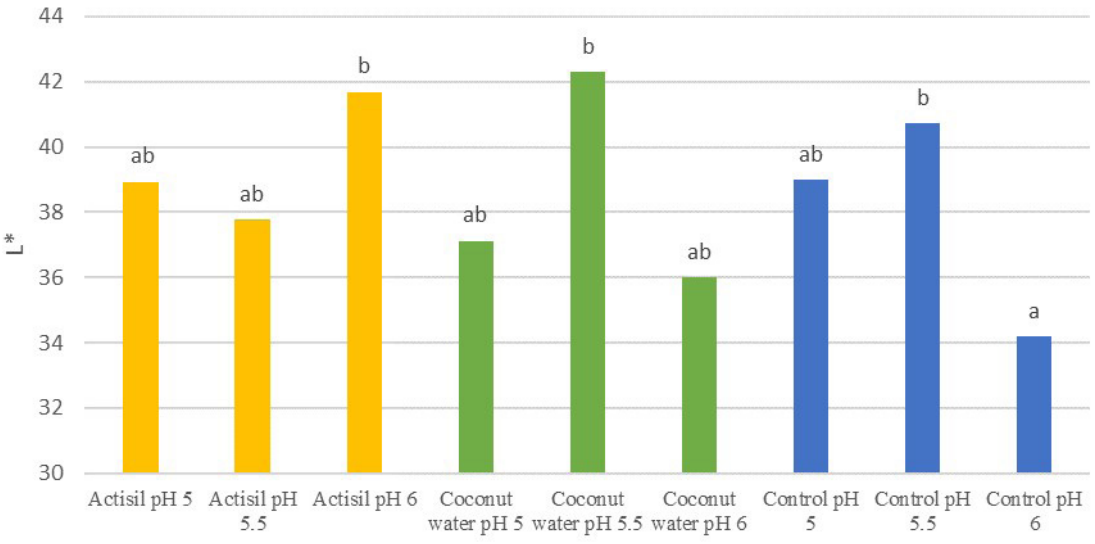

Fig. 4. Effect of 200 mg dm^{-3} Hydroplus™ Actisil and 15% coconut water (CW) in the WPM medium with different pH as a control on leaves colour using CIE *L*a*b** system, *L** the lightness coefficient at the end experiment. The figure: indicate x-axis – combinations of WPM medium, n = 32 shoots per treatments

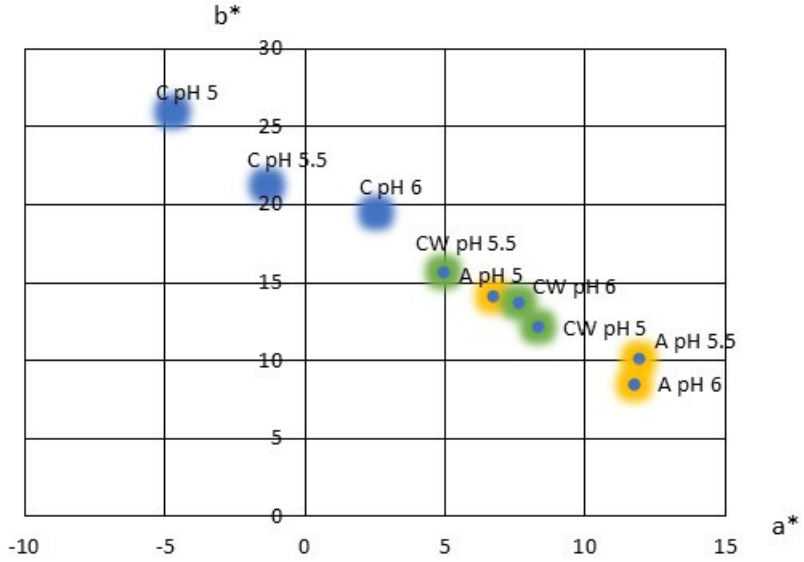

Fig. 5. Effect of 200 mg dm^{-3} Hydroplus™ Actisil (A) and 15% coconut water (CW) in the WPM medium with different pH as a control (C) on leaves colour using CIE *L*a*b** system, *a** (green colour) and *b** (yellow colour) at the end experiment, n = 32 shoots per treatments

for tracking the colour changes [Ochmian et al. 2010]. According to Krupa-Małkiewicz et al. [2019], the change in the parameter *L** is usually related to physiological attributes of the visual appearance of brightness. However, the highbush blueberry leaves grown on WPM medium pH 6.0 were the darkest (34.19). In the case of leaf colouration from the other combinations of the experiment, the differences were slight. The value of *a** parameter (colour ranging from green to red) determined on the surface of leaf ranges from –4.71 (control, pH 5.0) to 11.90 (WPM + 200 mg dm^{-3} Actisil, pH 5.5) (Fig. 5). It was observed that as the pH of the medium increased, the value of the parameter *a** increased. Opposite results were observed for the parameter *b** (colour ranging from yellow to blue), ranging from 25.98 (control, pH 5.0) to 8.52 (WPM + 200 mg dm^{-3} Actisil, pH 6.0) (Fig. 5). The addition of Actisil solution or coconut water to the WPM medium affected the colour of the highbush blueberry leaves as compared to the control group. Whereby, the leaves of highbush blueberry grown on WPM medium with addition of 15% CW were greener and less yellow than leaves grown on WPM medium with Actisil.

In the studies of Krupa-Małkiewicz and Calomme [2021], the application of Actisil in MS medium, regardless of concentration, had a positive influence on the intensity of the green and yellow colours of petunia leaves *in vitro*. According to Barbosa et al. [2015], increasing chlorophyll concentration in leaves can improve light interception and better performance of photosynthetic parameters.

CONCLUSIONS

In conclusion, this study has shown that micropropagation of *V. corymbosum* cv. Liberty can be enhanced by supplementing the culture medium with 200 mg dm^{-3} Hydroplus™ Actisil solution as recommended by the producer. Moreover, results have shown that using coconut water as a natural complex of organic substances influences plant height and number of new shoots and can replace zeatin. Maximum vegetative development of blueberry explants was obtained at pH 5. Commercial Si solution (Hydroplus™ Actisil) can be successfully used as an agent in blueberry *in vitro* culture, strongly stimulating plant growth and

reducing stress at higher pH of the medium. Coconut water did not alleviate the negative effects of the higher pH of the WPM medium in micropropagation of highbush blueberry.

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