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Original Article

The Effects of Synthetic Auxin and a Seaweed-based Biostimulator on Physiological Aspects of Rhizogenesis in Ninebark Stem Cuttings

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Abstract

Recently, a trend can be observed to replace plant protection chemicals in nursery production by products of natural origin. Such products increase plants' resistance to stressful conditions and lower their susceptibility to pathogens. They may also offer an alternative to chemicals stimulating rhizogenesis. The aim of this work was to evaluate the effects of foliar applications of AlgaminoPlant, a biostimulator based on a seaweed extract, relative to auxin-containing preparations, on root growth in stem cuttings of common ninebark (*Physocarpus opulifolius*). Cuttings were sprayed with water solution of IBA or AlgaminoPlant once, twice or three times during the rooting period at one week intervals and powder IBA was also applied directly to the bases of cuttings. The best rooting was observed after powder IBA application and satisfactory results were observed for AlgaminoPlant and aqueous IBA solution. Treatments with AlgaminoPlant and IBA increased photosynthetic efficiency, chlorophyll contents, total soluble sugars, soluble proteins, indole derivatives and catalase activity while the hydrogen peroxide level and peroxidase activity were reduced. The effects of AlgaminoPlant on rhizogenesis were comparable to synthetic IBA, or only slightly lower. Hence, if needed, synthetic auxin IBA may be replaced by AlgaminoPlant which is considered an environmentally friendly product.

Keywords: AlgaminoPlant, IBA, organic compounds, photosynthesis, rooting

Introduction

Production of plant material in the EU is controlled by stringent environmental, economic, juridical and social regulations. To mitigate possible adverse environmental effects, serious restictions have been placed on various compounds used in horticultutal production that contain synthetic auxins and various plant protection chemicals (Ludwig-Muller, 2000). This stimulates interest in substitute preparations that are environmentally friendly yet still effective in rhizogenesis and in successful acclimation of plants to different conditions in successive steps of plant production (Wojdyła, 2004; Pacholczak *et al.*, 2012).

Biostimulators may constitute such a group of compounds as they contain natural substances with little, if any, predicted environmental effect. Recently they are often considered as fertilizers, rather than plant protection substances, which simplyfies the registration procedure and eliminates the time required to classify them into specific groups of chemicals. Prior to the entry on a list of approved products they have to undergo toxicity tests. They can be used only under certain strict registration requirements (Dąbrowski, 2008).

Biostimulators derived from sea weed species such as Kelpak (PUH Chemirol, Poland), Goëmar (Arysta LifeScience, Poland), Pentakeep-V (Agroniwa, Poland) might be an alternative to chemicals banned by EU (Dobrzański *et al.*, 2008). The mechanisms of their action are poorly understood. Consequently, a series of experiments have been undertaken to to determine their efficiency and efficacy. Such information is essential for nurserymen. The positive effects of biostimulators on ornamental shrub propagation have already been reported (Shevchenko, 2008; Szabó and Hrotkó, 2009; Pacholczak *et al.*, 2010; 2012).

In this study, a new biostimulator, AlgaminoPlant (Varichem, Poland) has been tested. It is a liquid preparation produced on the base of a seaweed extract (18%) from *Sargassum*, *Laminaria*, *Ascophyllum*, and *Fuscus*. It contains phytohormones whose gibberellin-like activity expressed in equivalents of gibberellic acid is equal to 0.005% gibberellic acid (GA_3), the cytokinin activity is equal to 0.0005% benzyladenine (BA), and the auxin-like activity corresponds to 0.003% indole-3-acetic acid (IAA). It is supplemented by potassium salts of amino acids at 10% (Matysiak *et al.*, 2010). The results of our earlier tests in propagation of woody plants look promising (Pacholczak *et al.*, 2012; 2013; Pacholczak and Pietkiewicz, 2014).

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According to Khan *et al.* (2009), seaweed extracts not only stimulate growth and development of a root system but they also increase the organic compound contents in cuttings. Gawrońska *et al.* (2008) and Borowski (2009) have shown that biostimulators are capable of increasing the chlorophyll contents in cuttings' leaves and stimulate gas exchange. Both, the green pigment level and the photosynthetic rate directly affect the carbohydrate contents with is responsible for the energetic status of plants. In vegetatively propagated plants the accumulation of carbohydrates, especially in the form of simple sugars, may be decisive for the success of rhizogenesis, especially in the very early stages of root regeneration (Costa *et al.*, 2007).

Reactive oxygen species (ROS) are a by-product of normal cell metabolism in plants; however, under stress conditions, the balance between their production and elimination is disturbed. ROS rapidly inactivate enzymes, damage vital cellular organelles and destroy membranes by inducing the degradation of pigments, proteins, lipids and nucleic acids (Karuppanapandian *et al.*, 2011). Their synthesis and accumulation may lead to changes in cell structure and function of cell structures. Wounding is a part of a cutting's preparation; hence, ROS are always present during rhizogenesis. Hydrogen peroxide is one of such ROS that may accumulate in plant vessels, stomata, accompanying cells and damaged tissues. It is formed in a spontaneous dismutation reaction of a superoxide radical with simultaneous oxygen production, or in a reaction catalyzed by the superoxide dismutase (Dietz *et al.*, 2006). Oxidoreductases are a specific group of enzymes which catalyze reactions of oxidation and reduction. Such reactions maintain homeostasis between oxidised and reduced form of a compound, such as oxygen (Hodges, 2001). When these processes are disturbed the ROS are formed and the oxidative stress occurs. The degree of plant sensitivity to such stress depends mainly on its ability to eliminate free radicals and less so, on the rate of their formation. The enzymes responsible for the removal of free radicals are the superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX). Little is known about the relationship between oxidative stress and rhizogenesis in cuttings. One may assume such stress hampers root formation but this has to be verified experimentaly (Neves *et al.*, 1998; Libik *et al.*, 2005).

Common ninebark (*Physocarpus opulifolius* Maxim.) is a shrub with decorative leaves, resistant to air pollution and low temperatures, therefore, it is highly desirable in urban greenery. It is often planted in parks, squares, in the green areas of the housing estates, as well as in the gardens (Dirr, 2009). It is propagated by stem cuttings and its cultivars are generally considered to be difficult to root.

The aim of the trials was to test the effects of AlgaminoPlant on rhizogenesis in common ninebark and to compare its effectiveness with the synthetic auxin IBA. Synthetic IBA has been routinely used by the nurserymen to stimulate rooting but its future use is threatened by a EU ban on synthetic plant hormones. An attempt was also made to detect relationships, if any, between the gas exchange rates and several biochemical parameters in cuttings, as influenced by treatments, and the subsequent rhizogenesis.

Materials and methods

Experimental design

The experiments were conducted in 2012 and 2013 in a commercial nursery of M.M. Kryt in Wola Prażmowska, on common ninebark (*Physocarpus opulifolius* Maxim. 'Red Baron'). Semi lignified one nodal stem cuttings were prepared and rooted in styrofoam boxes. Cuttings were inserted to the depth of 2 cm into a mixture of peat and perlite (2:1 v/v), pH 5.0. The mixture was thoroughly wetted and pressed, and covered with 0.5 cm layer of coarse sand. Biostimulator AlgaminoPlant was used in aqueous solution (0.2%), on cuttings' leaves. Its effects were compared to those of the synthetic auxin (indole-3-butyric acid, IBA). IBA was applied either directly to the bases of cuttings in the form of the commercially available rooting powder Rhizopon AA (2% IBA), or by spraying cuttings with aqueous solution of 200 mg·l^{-1} (0.984 mmol·l^{-1}) IBA. Control cuttings were sprayed with distilled water. Spraying was done using a hand pressure sprayer (1 litre volume) at the beginning of the experiment (June 29 2012 and June 28 2013), and repeated twice at one week intervals, on July 6 and July 13 in 2012 and July 5 and July 12 in 2013. Cuttings were completely coverd with the solutions until drop off. Rooting took place in plastic tunnels equipped with automatic watering, mist systems and shading devices. The conditions during rooting were: temperature 25-28 °C and relative humidity 90-100%. During the first two weeks, the cuttings were protected against sun with an opaque foil and a shading cloth.

The experiment consisted of six treatments (Table 1), each in three replications, each replication containing 20 cuttings. Percentages of rooted cuttings and the degree of rooting were determined 8 weeks after the start of the experiment. The degree of rooting was evaluated on a 5-point scale rating the development of the root ball (Table 2).

Photosynthesis and respiration rate

On July 7, after the third spraying with AlgaminoPlant the following parameters of gas exchange were determined in the cuttings:

- P_n – net photosynthetic rate (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)
- R_d – respiration rate (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)

The parameters of gas exchanged were measured by the CIRAS-2 gas analyzer (PP System Inc., Amesbury, MA, USA). The measurements were done on three cuttings from each of three replications (nine records for each data point), at noon, under natural photosynthetic photon flux of 1400-1600 μmol·m^{-2}·s^{-1} at 25 °C and CO_2 concentration 320-380 μmol mol^{-1} air.

Table 1. A list of treatments in the experiment

No. of treatment	Methods of cuttings treatment
1	Control '0' 1 spraying with distilled water (H_2O)
2	Rhizopon AA (2% IBA) powder
3	1 spraying with IBA 200 mg ·l^{-1}
4	1 spraying with AlgaminoPlant 0.2%
5	2 sprayings with AlgaminoPlant 0.2%
6	3 sprayings with AlgaminoPlant 0.2%

Table 2. Evaluation scale of the root development

Characteristic of the degree of rooting	Score
Cutting without visible roots	1
A few (1-3) short roots	2
4 - 5 roots, some of them branched, no root ball formed	3
Medium sized root system composed of 6 – 10 branched roots forming a root ball	4
Well developed, branched root system forming a root ball (over 10 roots)	5

Table 3. The effect of IBA and AlgaminoPlant on degree and percentage of rooting cuttings of *Physocarpus opulifolius* 'Red Baron'

Treatment	Year	Control	Rh. AA 2% IBA powder	IBA 200 $mg{\cdot}l^{-1}$ spray	AlgaminoPlant 0.2% spray		
					× 1 time	× 2 times	× 3 times
Rooting degree	2012	1.8±0.2 a*	4.7±0.1 d	3.9±0.2 c	2.2±0.1 a	2.9±0.1 b	2.2±0.3 a
	2013	2.0±0.1 a	3.6 ±0.1d	2.9±0.2 b	3.2±0.1 bc	3.4±0.1 cd	3.1±0.1 bc
Rooting percentage	2012	46.7±5.1a*	100.0±0.2 d	96.7±3.2 d	53.3±3.8 b	56.7±3.0 b	73.3±3.2 c
	2013	48.3±1.6 a	81.7±3.2 c	61.7±1.6 b	61.1±3.2 b	65.0±1.1 b	65.0±1.1 b

The conditions during measurements were: temperature 20-25 °C and relative humidity 80-90%. The respiration rate was determined after shading the measurement chamber and extinction of photosynthesis. The leaves were allowed to come to equilibrium in the chamber. When measured with an infrared gas analyzer, the negative photosynthetic rate is equal to the respiration rate.

Biochemical analysis

Leaves from 20 cuttings per treatment were collected three weeks after the beginning of the experiment, from cuttings sprayed with IBA, sprayed 3-times with AlgaminoPlant and control cuttings sprayed with water. They were finely chopped, mixed, and 0.5 g samples were used for the measurements. Triplicate extracts were prepared for each analysis and three measurements were done for each extract producing nine readings for each data point.

For the dry matter content, 1 g samples were dried at 105 °C to constant weight. The total chlorophyll content (chlorophyll *a*+*b*) was determined according to Lichtenthaler and Wellburn (1983). Total soluble sugars were determined according to Dubois *et al.* (1956) and soluble proteins according to Lowry *et al.* (1951). The indole derivative contents were measured by the Gordon-Weber's colorimetric method (Gordon and Weber, 1951) with the Salkowski reagent (Ehmann, 1977) and expressed as IAA equivalents. Polyphenolic acids were measured by the colorimetric method with the Arnow's reagent according to the Polish Norm PN-91/R-87019. The hydrogen peroxide content was measured by the method of Zhang *et al.* (2013). The catalase activity was analyzed according to Goth (1991), and the peroxidase activity – by the method of Nakano and Asada (1981).

Statistical analysis

Arcsine transformation was performed for all data taken in percentages (Snedecor and Cochran, 1967). To compare the means, percentages of rooted cuttings were transformed according to Bliss. All data - percentages of rooted cuttings and the results of biochemical analyses were subjected to the one-factorial ANOVA followed by Newman-Keuls test at $p < 0.05$ (Wójcik and Laudański, 1989).

Results and Discussion

The experiments were carried out in a plastic tunnel adequately equipped to control the inside climate under normal conditions. However, the summer in 2013 was unusually hot and the temperature inside the tunnel could not always be maintained at the optimal level. This might be the reason for weaker rooting in 2013 as compared to 2012 and differences in the cuttings' responses to treatments in both years.

Rooting of cuttings

Rooting of cuttings depends on many factors of which the most important are the species-specific ability to totipotency and regeneration (Hartmann *et al.*, 2002; Spethmann, 2007). In the difficult-to-root species this ability is low so rhizogenesis must be enhanced. Until recently, this has been done by auxins which mechanism of action is well known (Spethmann, 1998). Recently, other rooting stimulators have been tested, such as extracts of seaweed (Shevchenko, 2008; Szabó and Hrotkó, 2009; Poşta and Hernea, 2011), citrus fruit or crustacea (Jacygrad and Pacholczak, 2010).

Experimental treatments significantly affected the degree and percentage of rooting in stem cuttings. In controls, the degree of rooting was similarly low in both years. The commercial rooting powder increased this parameter 2.6 and 1.8 - fold relative to control in 2012 and 2013, respectively (Table 3). Also, foliar application of IBA improved the root ball development, especially in 2012. Rhizopon AA was the most efficient treatment increasing the rooting percentage to 100% and 82% in 2012 and 2013, respectively. In the first year, the aqueous IBA solution was equally effective as the powder while in the second year, the rooting percentage was increased to a lower degree. Positive effects of Rhizopon and IBA on rhizogenesis in ninebark cultivars were earlier reported by Jacygrad and Pacholczak (2010) as well as by Pacholczak *et al.* (2013). Also Pirlak (2000) confirmed that the most efficient auxin in rooting of dogwood stem cuttings was IBA but in the concentration 60 $mg{\cdot}l^{-1}$, considerably lower than in our trials on *P. opulifolius.*

Double application of AlgaminoPlant improved the degree of rooting in both years, but in 2013 also two other dosages of the biostimulator significantly affected root ball development and produced results comparable to the auxin. All treatments of the biostimulator improved the percentage of rooting percentage relative to control; the threefold application was the best. In 2013 a single, double and triple spraying were equally effective, producing results comparable to the foliar auxin application (Table 3). Similar observations were done in two dogwood cultivars (Pacholczak *et al.*, 2012). Positive effects of AlgaminoPlant on root growth in carrot and parsley were also reported by Dobrzański *et al.* (2008).

Photosynthesis and the respiration rate

The photosynthesis rate ranged between 2.0 and 8.8 µmol $CO_2{\cdot}m^{-2}{\cdot}s^{-1}$, being higher in 2013 than in 2012 (Table 4). Most treatments increased photosynthesis relative to respective controls, with the foliar application of IBA being the most efficient. Rhizopon AA gave a significantly lower increase in the photosynthetic rate than the IBA water solution. In 2012 a single or double spraying with AlgaminoPlant produced slightly smaller effects than spraying with the auxin; in 2013 they were similar to both treatments with IBA. In 2013 both auxin

Table 4. The effect of IBA and AlgaminoPlant on photosynthetic and respiration rates (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) of cuttings of *Physocarpus opulifolius* 'Red Baron'

Treatment	Year	Control	Rh. AA 2% IBA powder	IBA 200 mg·l^{-1} spray	AlgaminoPlant 0.2% spray		
					× 1 time	× 2 times	× 3 times
Photosynthetic rates	2012	2.0±0.1 a*	2.3±0.1 ab	3.9±0.3 e	3.1±0.1 cd	3.4±0.3 cd	2.0±0.1 a
	2013	6.6±0.3 a	7.6±0.1 b	8.8±0.2 c	8.4±0.1 bc	7.9±0.1 bc	6.5±0.4 a
Respiration rates	2012	1.0±0.2 ab*	1.0±0.2 ab	0.8±0.1 a	1.3±0.1 b	1.0±0.1 ab	0.8±0.1 a
	2013	3.1±0.3 c	2.2±0.4 a	2.6±0.2 b	3.2±0.3 c	3.3±0.3 c	2.6±0.1 b

treatments and the triple application of AlgaminoPlant reduced the respiration rate relative to control. According to Wrochna *et al.* (2008) biostimulators can affect the photosynthesis rate by changing the electron flow. One of the observed effects of AlgaminoPlant was enhanced photosynthesis in cuttings; a similar effect was described in the yellow ninebark cultivar 'Dart's Gold' (Pacholczak and Pietkiewicz, 2014). Photosynthesis is reduced under stressful conditions but our results show that foliar application of a biostimulator may alleviate this phenomenon. Such an effect would be especially important for cuttings which remain under water stress until they form a new root system (Elsheery and Cao, 2008).

Biochemical analysis

According to Kranner *et al.* (2003) changes in the chlorophyll contents can be regarded as a defense response to stressful conditions. The chlorophyll *a+b* levels in control cuttings were 7.2 and 6.5 mg·g^{-1} DW in 2013 and 2012, respectively (Table 5). In the first year a small but statistically significant increase in chlorophyll content occurred after foliar application of both IBA and AlgaminoPlant, while in 2013 only the latter increased the chlorophyll level, by 46% relative to the control. A similar increase in green pigment after a biostimulator treatment was earlier reported for dogwood cuttings (Pacholczak *et al.*, 2012), wheat (Vician and Kováčik, 2013) and tomato (Mikiciuk and Dobromilska, 2014).

Carbohydrates are a resource for respiration providing the energy indispensable for regeneration and growth of the root system. In 2012, the total soluble sugars in control cuttings were about 30% lower than in 2013; treatments with IBA and AlgaminoPlant increased these levels by 67% and 82%, respectively (Table 5). Agulló-Antón and Sánchez-Bravo (2011) observed an over twofold increase in soluble sugars in cuttings of *Dianthus caryophyllus* treated with auxins. A significant rise in total soluble sugars after the application of a biostimulator based on sea weeds was reported by Sivasankari *et al.* (2006) in *Vigna sinensis* and by Rathore *et al.* (2009) in soya.

Foliar application of IBA or AlgaminoPlant resulted in significant increases in soluble protein contents in cuttings in both years (Table 5). The effects of both treatments were similar in 2012 while in 2013 the biostimulator was more effective: 54% increase in proteins after the AlgaminoPlant treatment *vs.* 17% for IBA. Proteins in cuttings affects rhizogenesis not only because they constitute the building material for new roots (Husen and Pal, 2007). Proteins located in the endoplasmic reticulum and cell membranes act as auxin receptors which enhance rooting (Günther and Scherer, 2011). Proteins in plant cells also include enzymes of the oxidative stress (Starke *et al.*, 1997; Davies, 2000). An increase in proteins following an application of the biostimulator Atonik™ was reported in cotton (Oosterhuis, 2008).

The amounts of indole derivatives in ninebark cuttings in this study were comparable in 2012 and 2013 (Table 5). In 2012 only AlgaminoPlant increased it by 10% relative to the control. In 2013 the effect of AlgaminoPlant was similar but the highest level of indole derivatives was determined in cuttings sprayed with the water IBA solution where it reached 158% of the level found in the untreated controls. This is in line with the reports of Scagel and Linderman (2001) who after IBA application found the 50% increase in endogenous auxins in douglas fir, 40% in lodgepole pine and 30% in Engelmann's spruce. Auxins, and especially the indoleacetic acid (IAA), are unstable and their concentration in a plant depends on a species (cultivar), stock plant age, cutting type, light, water, etc. (Davies, 1995).

Polyphenolic acids participate in plant response to the oxidative stress and in the general defense system against stressful conditions (Diáz *et al.*, 2001; Michalak, 2006). In 2012 the polyphenolic acids content in control cuttings was about 28% lower than in 2013 (Table 5), again probably reflecting the major summer temperature difference. Foliar application of IBA reduced the polyphenolic acid content by 13% in 2012; in the second year there was an increase by 17%. The AlgaminoPlant treatment produced a 10% increase of polyphenolic acid in 2013 though no such increase occurred in 2012. Similar increase was reported by Jacygrad and Pacholczak (2010) in ninebark cuttings sprayed with a biostimulator AminoTotal. In tomato and sweet pepper, biostimulators Asahi SL i Biochicol 020 reduced the levels of polyphenolic acids relative to untreated controls (Cwalina-Ambroziak and Amarowicz, 2012). Basak *et al.* (1995) observed a drop in polyphenolic acids resulting from application of the mixture of IAA and IBA: from 15% in cuttings of *Cynometra iripa*, *Heritiera fomes* and *Thespesia populnea* to 40% in *Bruguiera parviflora*.

The hydrogen peroxide levels in the cuttings were comparable in both years (Table 5). Treatments tended to lower their values: in 2012 only the AlgaminoPlant reduced the H_2O_2 contents while in 2013 the H_2O_2 contents were reduced by both, IBA and AlgaminoPlant, by 10% and 15%, respectively. According to Zhu *et al.* (2012) such a decrease may limit stress effects. It may result from increased catalase activity. Catalase is known to be responsible for alleviating stress due to excessive humidity or drought (Yuchuan *et al.*, 2013). The latter occurs during rooting of cuttings. The activity of catalase was by about 15% higher in 2013 than in 2012. In both years both treatments increased it significantly, by 15-20% relative to the respective untreated control. According to Zhang *et al.* (2006), extracts from algae *Ascophyllum nodosum* increased of catalase activity in *Agrostis stolonifera*.

The activity of peroxidase was about 35% higher in 2013 than in 2012 (Table 5). In 2012 the foliar IBA application lowered it by 26%; in 2013 both treatments reduced it, by 13% and 10%, respectively. According to Costa *et al.* (2013)

Table 5. The effect of IBA spray and AlgaminoPlant (treated 3 times) on total chlorophyll, total sugar soluble proteins and polyphenolic acids (mg·g^{-1} DW), indole derivatives (µg IAA·g.$^{-1}$ DW), hydrogen peroxide (H_2O_2) (µg·g^{-1} DW), catalase and peroxidase activity (mkat·g^{-1} DW) in cuttings of *Physocarpus opulifolius* 'Red Baron'

Treatment / content	Season	Control	IBA 200 mg·l^{-1} spray	AlgaminoPlant 0.2% 3 times spray
Chlorophyll	2012	7.2 ±1.1 a*	7.9 ±1.1 bc	8.1 ±1.2 c
	2013	6.5 ±1.1 a	5.9 ±1.1 a	9.5 ±1.1 c
Total sugar	2012	68.8 ±4.3 a*	115.1 ±3.1 b	125.1 ±6.1 c
	2013	101.6 ±3.1 a	133.2 ±6.1 b	131.3 ±9.1 b
Soluble proteins	2012	3.7 ±0.5 a*	4.6 ±0.8 b	5.0 ±0.5 b
	2013	3.5 ±0.1 a	4.1 ±0.4 b	5.4 ±0.8 c
Indole derivatives	2012	87.4 ±2.4 a*	91.7 ±3.6 ab	94.7 ±1.6 b
	2013	88.5 ±5.1 a	139.4 ±7.4 c	99.4 ±7.9 b
Polyphenolic acids	2012	8.1 ±1.9 b*	5.5 ±1.6 a	8.1 ±1.3 b
	2013	11.3 ±2.1 a	13.2 ±2.4 c	12.4 ±2.6 b
Hydrogen peroxide	2012	337.0 ±4.3 b*	326.3 ±5.1 b	294.4 ±7.1 a
	2013	378.8 ±9.7 c	342.1 ±8.3 b	321.7 ±8.9 a
Catalase activity	2012	1615.8 ±15.5 a*	1815.5 ±13.7 b	1857.7 ±15.1 b
	2013	1863.0 ±10.1 a	2262.5 ±13.5 b	2271.3 ±10.1 b
Peroxidase activity	2012	0.888 ±0.06 b*	0.655 ±0.01 a	0.778 ±0.03 b
	2013	1.201 ±0.05 b	1.045 ±0.01 a	1.078 ±0.01 a

peroxidase activity depends on a developmental phase of a cutting and mother plant nutritional status, both in terms of minerals and carbohydrates, as well as on sink establishment at cutting bases. The decrease in activity of peroxidase would be relevant to generate the necessary high level of auxin and H_2O_2 required for adventitious root induction (Li *et al.*, 2009).

These results can be treated as a starting point for studies on alternative methods of rooting of cuttings with the use of biostimulators instead of auxins. Such studies should be carried out on different species to verify and confirm relationships between certain physiological processes and rhizogenesis what could allow either to replace auxins by biostimulators or to lower their doses in a joint application with biopreparations.

Conclusions

The study has revealed that Rhizopon AA containing IBA and the water solution of IBA stimulate formation of root system in ninebark stem cuttings. The applications of biostimulator AlgaminoPlant produce comparable or slightly weaker effects to the treatments with synthetic IBA. Treatments enhancing rhizogenesis affect physiological processes in ninebark cuttings.

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References

Agulló-Antón MA, Sánchez-Bravo J (2011). Auxins or sugars: What makes the difference in the adventitious rooting of stored carnation cuttings? Plant Growth Regulation 30:100-113.

Basak UC, Das AB, Das P (1995). Metabolic changes during rooting in stem cuttings of five mangrove species. Plant Growth Regulation 17:141-148.

Borowski E (2009). Response to chilling in cucumber (*Cucumis sativus* L.) plants treated with triacontanol and Asahi SL. Acta Agrobotanica 62:165-172.

Borowski E (2009). Response to chilling in cucumber (*Cucumis sativus* L.) plants treated with triacontanol and Asahi SL. Acta Agrobotanica 62:165-172.

Costa JM, Heuvelink E, Pol PA, Put HMC (2007). Anatomy and morphology of rooting in leafy rose stem cuttings and starch dynamics following severance. Acta Horticulturae 751:495-502.

Costa CT, Almeida MR, Ruedell CM, Maraschin JS, Fett-Neto AG (2013). When stress and development go hand in hand: main hormonal controls of adventitious rooting in cuttings. Frontiers in Plant Science 4:133. doi:10.3389/fpls.2013.00133.

Cwalina-Ambroziak B, Amarowicz R (2012). Effects of biological and fungicidal environmental protection on chemical composition of tomato and red pepper fruits. The Polish Journal of Environmental Studies 21(4):831-836.

Davies PJ (1995). The plant hormones: their nature, occurrence and functions. In: Davies PJ (Ed). Plant Hormones Physiology, Biochemistry and Molecular Biology. Kluwer Academic Publishers, Dordrecht, The Netherlands pp 1-12.

Davies KJA (2000). Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. International Union of Biochemistry and Molecular Biology Life 50:79-289.

Dąbrowski Z (2008). Field Crops. Preface. In: Dąbrowski Z (Ed.). Biostimulators in modern agriculture. Wieś Jutra, Warsaw, Poland pp 5-6.

Diáz J, Bernal A, Pomar F, Merino F (2001). Induction of shikimate dehydrogenase and peroxidase in pepper (*Capsicum annum* L.) seedlings in response to copper stress and its relation to lignification. Plant Science 161:179-188.

Dietz KJ, Jacob S, Oelze ML, Laxa M, Tognetti V, Nunes de Miranda SN, Baier M, Finkmeier I (2006). The function of peroxiredoxins in plant organelle redox metabolism. Journal of Experimental Botany 57:1697-1709.

Dirr MA (2009). Manual of woody landscape plants. Stipes Publishing L.L.C. (6th ed), Champaign pp 1324-1325.

Dobrzański A, Anyszka Z, Elkner K (2008). Reakcja marchwi na ekstrakty pochodzenia naturalnego z alg z rodzaju Sargassum – AlgaminoPlant i z leonardytu – HumiPlant. [Carrot response to natural extracts from *Sargassum* algae – AlgaminoPlant and from leonardit – HumiPlant]. Journal of Research and Applications in Agricultural Engineering 53:53-58 (in Polish).

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28:350-356.

Ehmann A (1977). The van Urk-Salkowski reagent - a sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. Journal of Chromatography 132:267-276.

Elsheery NI, Cao KF (2008). Gas exchange, chlorophyll fluorescence, and osmotic adjustment in two mango cultivars under drought stress. Acta Physiologiae Plantarum 30:769-777.

Gawrońska H, Przybysz A, Szalacha E, Słowiński A (2008). Physiological and molecular mode of action of Asahi SL biostimulator under optimal and stress conditions. In: Gawrońska H (Ed). Biostimulators in modern agriculture: general aspects. Wieś Jutra, Warsaw, Poland pp 54-76.

Gordon SA, Weber RP (1951). Colorimetric estimation of indoleacetic acid. Plant Physiology 26:192-195.

Goth L (1991). A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta 196:143-152.

Günther F, Scherer E (2011). Auxin-Binding-Protein1, the second auxin receptor: what is the significance of a two-receptor concept in plant signal transduction? Journal of Experimental Botany 62(10):3339-3357.

Hartmann HT, Kester DE, Davies FT, Geneve RL (2002). Plant propagation. Principles and practices. Prentice Hall, Upper Saddle River (7th ed), New Jersey.

Hodges DM (2001). Chilling effects on active oxygen species and their scavenging systems in plants. In: Basra A (Ed). Crop responses and adaptations to temperature stress. Food Products Press, Binghamton pp 53-78.

Husen A, Pal M (2007). Metabolic changes during adventitious root primordium development in *Tectona grandis* Linn. f. (teak) cuttings as affected by age of donor plants and auxin (IBA and NAA) treatment. New Forests 33:309-323.

Jacygrad E, Pacholczak A (2010). Effect of biopreparations AminoTotal and Biochikol 020 PC on rhizogenesis in stem cuttings of *Physocarpus opulifolius* 'Dart's Gold' and 'Diabolo'. Annals of Warsaw Agricultural University, Horticulture Landscape Architecture 31:19-28.

Karuppanapandian T, Moon JC, Kim C, Manoharan K, Kim W (2011). Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Australian Journal of Crop Science 5(6):709-725.

Khan W, Rayirath UP, Subramanian S, Jitesh MN, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie J, Prithiviraj B (2009). Seaweed extracts as biostimulants of plant growth and development. Journal of Plant Growth Regulation 28:386-399.

Kranner I, Zorn M, Turk B, Wornik S, Beckett RP, Batič F (2003). Biochemical traits of lichens differing in relative desiccation tolerance. New Phytologist 160(1):167-176.

Lichtenthaler HK, Wellburn AR (1983). Determinations of total carotenoids and chlorophylls a and b leaf extracts in different solvents. Biochemical Society Transaction 603:591-592.

Li SW, Xue L, Xu S, Lizhe A (2009). IBA-induced changes in antioxidant enzymes during adventitious rooting in mung bean seedlings: the role of H_2O_2. Environmental and Experimental Botany 66(3):442-450.

Libik M, Konieczny R, Pater B, Ślesak I, Miszalski Z (2005). Differences in the activities of some antioxidant enzymes and H_2O_2 content during rhizogenesis and somatic embryogenesis in callus cultures of the ice plant. Plant Cell Reports 23:834-841.

Lowry JOH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry 193:265-267.

Ludwig-Muller J (2000). Indole-3-butyric acid in plant growth and development. Plant Growth Regulation 32:219-230.

Matysiak K, Kaczmarek S, Kierzek R, Kardasz P (2010). Effect of seaweeds extracts and humic and fulvic acids on the germination and early growth of winter oilseed rape (*Brassica napus* L.). Journal of Research and Applications in Agricultural Engineering 55:28-32.

Michalak A (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies 15(4):523-530.

Mikiciuk M, Dobromilska R (2014). Assessment of yield and physiological indices of small-sized tomato cv. 'Bianka F_1' under the influence of biostimulators of marine algae origin. Acta Scientiarum Polonorum, Hortorum Cultus 13(1):31-41.

Nakano S, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiology 22:867-880.

Neves C, Sa MC, Amancio S (1998). Histochemical detection of H_2O_2 by tissue printing as a precocious marker of rhizogenesis in grapevine. Plant Physiology and Biochemistry 36:817-824.

Oosterhuis DM (2008). Atonik ™ biostymulator zwiększający zawartość azotu i białka w uprawie bawełny. [Atonik™ biostimulator increases the nitrogen content and protein crops of cotton]. In: Gawrońska H (Ed). Biostimulators in modern agriculture: book of abstracts. Wieś Jutra, Warsaw, Poland: 18 (in Polish).

Pacholczak A, Szydło W, Pijus J (2010). Effect of preparation Asahi SL on rhizogenesis in stem cuttings of *Physocarpus opulifolius* 'Dart's Gold' and 'Diabolo'. Annals of Warsaw Agricultural University, Horticulture Landscape Architecture 31:11-18.

Pacholczak A, Szydło W, Jacygrad E, Federowicz M (2012). Effect of auxins and the biostimulator AlgaminoPlant on rhizogenesis in stem cuttings of two dogwood cultivars (*Cornus alba* 'Aurea' and 'Elegantissima'). Acta Scientiarum Polonorum, Hortorum Cultus 11:93-103.

Pacholczak A, Szydło W, Petelewicz P, Szulczyk K (2013). The effect of AlgaminoPlant on rhizogenesis in stem cuttings of *Physocarpus opulifolius* 'Dart's Gold' and 'Red Baron'. Acta Scientiarum Polonorum, Hortorum Cultus 12:105-116.

Pacholczak A, Pietkiewicz S (2014). Gas exchange during rhizogenesis in common ninebark stem cuttings treated with biostimulators. Propagation of Ornamental Plants 14(2):90-96.

Pirlak L (2000). Effects of different cuttings times and IBA doses on the rooting rate of hardwood cuttings of cornelian cherry (*Cornus mas* L.). Journey of the Aegean Agricultural Research Institute 10(1):122-134.

Poşta DS, Hernea C (2011). The influence of rooting biostimulators and substrate on biometric characteristics of *Kerria japonica* (L.) DC seedlings. Journal of Horticulture, Forestry and Biotechnology 15:88-91.

Rathore SS, Chaudhary DR, Boricha GN, Gosh A, Bhatt BP, Zadope ST, Patolia JS (2009). Effect of seaweed extract on growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. South African Journal of Botany 75:351-355.

Scagel CF, Linderman RG (2001). Modification of root IAA concentration, tree growth, and survival by application of plant growth regulating substances to container-grown conifers. New Forests 21:159-186.

Sivasankari S, Venkatesalu V, Anantharaj M, Chandrasekaran M (2006). Effect of seaweed extracts on the growth and biochemical constants of *Vigna sinensis*. Bioresource Technology 97:1745-1751.

Shevchenko Y (2008). Effects of biostimulators on growth and physiological reactions of vegetables - tested on cucumber (*Cucumis sativus* L.). PhD Thesis, Humboldt-Universität, Berlin.

Snedecor GW, Cochran WG (1967). Statistical methods. The Iowa State University Press, Ames, Iowa USA pp 593.

Spethmann W (1998). Factors affecting rooting of difficult-to-root plants. Combined Proceedings International Plant Propagators' Society 48:200-205.

Spethmann W (2007). Increase of rooting success and further shoot growth by long cuttings of woody plants. Propagation of Ornamental Plants 7(3):160-166.

Starke DW, Chen Y, Bapna CP, Lesnefsky EJ, Mieyal JJ (1997). Sensitivity of protein sulfhydryl repair enzymes to oxidative stress. Free Radical Biology and Medicine Journal 23(3):373-84.

Szabó V, Hrotkó K (2009). Preliminary results of biostimulator treatments on *Crataegus* and *Prunus* stockplants. Bulletin UASVM Horticulture 66:223-228.

Vician M, Kováčik P (2013). The effect of folic application of MG-Titanit fertilizer on phytomass, chlorophyll production and the harvest of winter wheat. Conference MendelNet, 20-21.11.2013, Brno pp 162-168.

Wojdyła AT (2004). Chitosan (Biochikol 020 PC) in the control of some ornamental foliage diseases. Communications in Agricultural and Applied Biology Sciences, Ghent University 69:705-715.

Wójcik AR, Laudański Z (1989). Planowanie i wnioskowanie statystyczne w doświadczalnictwie. [Statistical planning and concluding in experimental works]. PWN, Warszawa (in Polish).

Wrochna M, Łata B, Borkowska B, Gawrońska H (2008). Effect of Asahi SL biostimulator on ornamental amaranth (*Amaranthus* spp.) plants exposed to salinity in growing medium. In: Gawrońska H (Ed). Biostimulators in modern agriculture: general aspects: ornamental and special plants. Wieś Jutra, Warsaw, Poland pp 15-30.

Yuchuan C, Wenhui Z, Xiaofeng W (2013). Physiological responses of *Quercus variabilis* seedlings to soil drought stress. Acta Botanica Boreali-Occidentalia Sinica 33(2):364-370.

Zhang Q, Fu S, Li H, Liu Y (2013). A nowel method for the determination of hydrogen peroxide in bleaching effluents by spectroscopy. BioResources 8(3):3699-3705.

Zhang Q, Zhang J, Shen J, Silva A, Dennis DA, Barrow CJ (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. Journal of Applied Phycology 8:445-450.

Zhu G, Yi Y, JianRong W, HuanCheng M, Yuan Z (2012). Effects of three rooting formula on rooting rate and protective enzyme activities in *Ceiba pentandra* cutting. Acta Agriculturae Universitatis Jiangxiensis 34(3):528-532.