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ORIGINAL RESEARCH PAPER in HORTICULTURAL PLANTS

The Effect of Biostimulators and Indole-3-Butyric Acid on Rooting of Stem Cuttings of Two Ground Cover Roses

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Abstract

One of the important groups of roses are ground cover roses, used not only as garden plants but also in urban green areas for covering scarps and hill slopes. Roses are mostly propagated in vitro and by budding, grafting, and cuttings however, the latter is feasible only in certain rose groups. To hasten the production of plant material, preparations that stimulate rhizogenesis are used, which are based mainly on auxins and enhance the growth of adventitious roots in cuttings. Other substances capable of stimulating plant growth are biostimulators, which can be an alternative to rooting formulations containing auxins. The goal of this experiment was to compare the effects of the biostimulators AlgaminoPlant and Goteo with that of the synthetic auxin indole-3-butyric acid (IBA) on rooting of cuttings in two ground cover roses Elfrid ('Kormuse') and Weisse Immensee ('Korweirim'). In the preparations, IBA in the form of Rhizopon (1% IBA) or water solution (200 mg L^{-1}) was used, whereas the biostimulators were applied at the concentration of 0.2% for foliage spraying. Both biostimulators positively affected the percentage of rooted cuttings, whereas their effect on the degree of rooting was comparable or slightly weaker than that of synthetic auxin. Both biopreparations stimulated the growth of new shoots in cuttings. The use of Goteo resulted in increased content of chlorophyll and total soluble sugars in cuttings, whereas levels of free amino acids and polyphenolic acids were decreased. The synthetic auxin IBA increased the total sugar concentration and the free amino acid contents in cuttings were decreased.

Keywords

shrub rose; growth regulator; seaweed preparation; propagation; rhizogenesis

1. Introduction

Roses belong to the family Rosaceae, which contains nearly 3,000 species (Gustavsson, 1999), 150 of which grow in Poland (Monder, 2007). It is a highly diversified and variable genus (Monder, 2007). In natural habitats, roses have been present for millions of years, occurring on all continents. However, Asia, in particular China, is considered their cradle (Meng et al., 2011). Garden roses have been classified into groups basing on morphological traits, species origins, and practical use. One of the systems, described by the American Rose Society, defines old roses as those that were known and cultured before 1867 and are now gathered into 21 groups, whereas modern roses contain 13 groups (Cairns, 2003). One of these groups is called the ground cover roses and contains those roses used not only in gardens but also in urban green areas (Gustavsson, 1999). Ground cover roses serve not only as ornamental plants but can also be used to stabilize scarps and hillside slopes, as well as for weed control. They are attractive because of their extensive blooming and are also easy to cultivate (Cairns, 2003).

Most frequently propagated rose cultivars are produced by budding or grafting, but the recommended propagation method for roses that grow well on their own roots is by cuttings. The easiest and most economical technique is to root two–three nodal leafy cuttings taken from flowering shoots (Wiśniewska-Grzeszkiewicz & Podwyszyńska, 2001). Rooting of roses is a long process during which cuttings are subjected to numerous environmental stresses (Ginova et al., 2012; Monder et al., 2014, 2016). To improve rose rooting success, growth regulators, especially auxins, such as IBA (indole-3-butyric acid) are commonly used (Cárderas-Navarro & López-Pérez, 2011; Hoşafçi et al., 2005; Kroin, 2014; Nasri et al., 2015).

Biostimulators may be an alternative to the auxin-based rooting enhancers (Monder et al., 2019; Pacholczak et al., 2016). They contain active substances of natural origin or microorganisms and when applied to plants, they stimulate life processes occurring at every level of biological organization. Biostimulators, such as Kelpak, Goëmar, AlgaminoPlant, Pentakeep-V, and Route and their effects have been intensively studied to determine if they are viable alternatives for the preparations, which are now banned from the market because of new regulations of the European Union (Dobrzański et al., 2008; du Jardin, 2015). However, knowledge of the effects of these aforementioned preparations is poor, except for the most popular biostimulator, Asahi SL (Gawrońska et al., 2008; Przybysz, 2010).

Mechanisms of the effects of biostimulators on physiology and biochemical changes they elicit in plants often remain unknown, primarily because of the diversity of raw materials used in their production (Ertani, 2015; du Jardin, 2015). The extracts from seaweeds affect not only the growth and development of root systems but also the contents of organic compounds in plants (Khan et al., 2009). The application of biostimulators results in increased concentration of photosynthetic pigments. This is closely associated with the plant's photosynthetic activity and carbohydrate levels because they affect the energetic state of the plant. For vegetative propagation, sugar accumulation may be crucial for rhizogenesis during the first stage of root regeneration (Costa et al., 2007).

AlgaminoPlant (Varichem, Poland) is a liquid preparation produced based on a seaweed extract (18%) from *Sargassum*, *Laminaria*, *Ascophyllum*, and *Fuscus*. It contains phytohormones whose gibberellin-like activity is expressed in equivalents of 0.005% gibberellic acid (GA_3), cytokinin activity equal to 0.0005% benzyladenine (BA), and auxin-like activity corresponding to 0.003% indole-3-acetic acid (IAA). It is supplemented by 10% potassium salts of amino acids (Matysiak et al., 2010).

The biostimulator Goteo (Arysta LifeScience, Poland) is a liquid preparation that stimulates the formation of a rhizogenous layer. It contains GA142, a biologically active filtrate from the seaweed *Ascophyllum nodosum*, 13% phosphorus as P_2O_5, and 5% potassium as K_2O. The recommended dosage for hastening the rooting system regeneration is a series of three–four treatments with a 0.2% solution every 2 weeks (Gajc-Wolska et al., 2012; Matysiak et al., 2010).

The goal of this study was to compare the effects of the biostimulators AlgaminoPlant and Goteo with those exerted by the synthetic auxin IBA on rooting of cuttings in two cultivars of ground cover roses Elfrid ('Kormuse') and Weisse Immensee ('Korweirim'). The auxin was applied either in the form of a commercial preparation, Rhizopon (1% IBA), or as an IBA water solution (200 mg L^{-1}).

2. Material and Methods

2.1. Rooting of Cuttings

The experiment began on June 22, 2018, in the commercial nursery M. M. Kryt in Wola Prażmowska. Two cultivars of ground cover roses were used, Elfrid ('Kormuse') and Weisse Immensee ('Korweirim'). The rooting of cuttings lasted 8 weeks and the experiment ended on August 20. Semilignified two–three nodal cuttings were harvested from 4-year-old stock plants growing in the field. They were then placed in Styrofoam boxes filled with a mixture of ground peat and coarse sand, 2:1 v/v, pH 4.9–5.1 and placed on beds under a plastic tunnel. During the first 2 weeks, the cuttings were protected from sunlight with an opaque foil and a shade cloth. Every week, cuttings were sprayed against Botrytis with 0.1% Topsin or Teldor. There were five treatments, each in three replicates, with each replicate containing 20 cuttings (Table 1). When applying Rhizopon AA, the cutting bases were first immersed into the powder preparation and the powder was excess shaken off. The solutions of the auxin IBA (200 mg L^{-1}) and biostimulators Goteo and AlgaminoPlant (0.2%) were applied using a hand sprayer until they run off the leaves.

Table 1 List of treatments in the experiment.

No. of treatment	Methods of cuttings' treatment
1	Control 0: single spraying with distilled water
2	Rhizopon AA (1% IBA) powder
3	Single spraying with IBA 200 mg L^{-1}
4	Single spraying with Goteo 0.2%
5	Single spraying with AlgaminoPlant 0.2%

After 8 weeks, the rooting of cuttings was evaluated on a 5-point scale rating the development of the root ball (Table 2). The percentage of rooted cuttings was also calculated and only the cuttings with root systems within the points of 2–5 were regarded as rooted and counted.

Table 2 Evaluation scale of root development.

Characteristics of the degree of rooting	Score
Cutting without visible roots	1
A few (one–three) short roots	2
Four–five 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

2.2. Biochemical Analyses

For biochemical analyses, leaves from 20 cuttings per treatment were sampled at the beginning and end of the experiment. They were finely chopped, mixed, and 0.5-g samples were used for the measurements. Extracts in triplicate were prepared for each analysis and three measurements were conducted for each extract resulting in nine readings for each data point.

The total chlorophyll content (chlorophyll a+b) was analyzed according to the method by Lichtenthaler and Wellburn (1983). Total soluble sugars were determined according to the method of Dubois et al. (1956) and free amino acids were measured by the method of Rosen (1957). Polyphenolic acids were measured by the colorimetric method with Arnow's reagent according to the Polish Norm PN-91/R-87019 (Polskie Towarzystwo Farmakologiczne, 1999).

2.3. Statistical Analyses

Percentage values were $arc\,\sin(x)1/2$ transformed according to Bliss normalize the data (Snedecor & Cochran, 1967). All data (degree of rooting, percentage of rooted cuttings, and results of biochemical analyses) were then analyzed using a one-factorial ANOVA followed by Newman–Keuls test at $\alpha = 0.05$ (Wójcik & Laudański, 1989). STATGRAPHICS Centurion XVI.I software (StatPoint Technologies, United States) was used for all statistical analyses.

3. Results

3.1. Rooting of Cuttings

One-factorial analysis of variance revealed a significant effect for all preparations on the degree of rooting and percentage of rooted cuttings in both rose cultivars. The weakest rooting occurred in the control treatments where the scores were 2.9 for Elfrid ('Kormuse') and 3.1 for Weisse Immensee ('Korweirim') (Table 3). The foliar application of AlgaminoPlant improved the result by 0.8, but only in the latter cultivar. A significant improvement in rooting was observed Goteo in both cultivars, and the biostimulator effects were comparable to that exerted by the synthetic auxin used either as Rhizopon AA or an IBA water solution (200 mg L^{-1}).

Table 3 The effect of IB, Goteo, and AlgaminoPlant on the degree of rooting in cuttings of *Rosa* Elfrid ('Kormuse') and Weisse Immensee ('Korweirim').

Cultivar	Control	Rh. AA	Spray 200 mg L^{-1} IBA	Spray Goteo 0.2% (single time)	Spray AlgaminoPlan 0.2% (single time)
Elfrid ('Kormuse')	2.9 a	3.5 c	3.3 bc	3.3 bc	3.1 b
Weisse Immensee ('Korweirim')	3.1 a	4.1 bc	4.4 c	4.1 bc	3.9 b

Means in a row followed by the same letter do not differ significantly at $p < 0.05$ ("a" is the lowest value).

The lowest percentage of rooted cuttings was obtained in the control treatments: 90.5% in Elfrid ('Kormuse') and 93.3% in Weisse Immensee ('Korweirim'). These values were increased by spraying cuttings with biostimulators and the application of IBA both as the water solution and the powder preparation of Rhizopon AA (Table 4).

Table 4 The effect of IB, Goteo, and AlgaminoPlant on the percentage of rooting in cuttings of *Rosa* Elfrid ('Kormuse') and Weisse Immensee ('Korweirim').

Cultivar	Control	Rh. AA	Spray 200 mg L^{-1} IBA	Spray Goteo 0.2% (single time)	Spray AlgaminoPlan 0.2% (single time)
Elfrid ('Kormuse')	90.5 a	96.7 b	96.7 b	95.3 b	95.0 b
Weisse Immensee ('Korweirim')	93.3 a	100.0 b	100.0 b	98.3 b	98.5 b

See Table 3 for notes.

3.2. Biochemical Analyses

The analysis of variance revealed a significant effect of preparations used in the experiment on the content of organic compounds in cuttings of both cultivars. An increase in chlorophyll relative to that of the control treatment was observed in rose cuttings sprayed with 0.2% water solutions of AlgaminoPlant and Goteo by 23% and 28% in Elfrid ('Kormuse') and by 19% in Weisse Immensee ('Korweirim'), respectively. In the latter cultivar, the chlorophyll content was also increased by nearly 20% by the foliar IBA application (Table 5).

Compared to that of the untreated cuttings, all of the rooting enhancers increased the total soluble sugar content in cuttings. The richest in total sugar content was the cuttings of Weisse Immensee ('Korweirim') sprayed with the water IBA solution, although this result was statistically comparable to the effects of the biostimulators. In Elfrid ('Kormuse'), the increase in total soluble sugar relative to the control (by 33%–39%) occurred by spraying with the solutions of Goteo, AlgaminoPlant, and Rhizopon (Table 5).

Generally, the levels of free amino acids in cuttings were reduced by the preparations stimulating rhizogenesis by an average of 33% in Elfrid ('Kormuse') and 15% in Weisse Immensee ('Korweirim') (Table 5). As compared to the control treatment, the application of AlgaminoPlant and Goteo resulted in a decrease in contents of

Table 5 The effects of IB, Goteo, and AlgaminoPlant on the chlorophyll (*a*+*b*) (mg g^{-1} dry weight; DW), total soluble sugar (mg g^{-1} DW), free amino acid (μmol g^{-1} leucine DW), and polyphenolic acid (mg g^{-1} DW) contents in cuttings of *Rosa* Elfrid ('Kormuse') and Weisse Immensee ('Korweirim').

Content	Cultivar	Control	Rh. AA	Spray 200 mg L^{-1} IBA	Spray Goteo 0.2%	Spray AlgaminoPlan 0.2%
Chlorophyll (*a*+*b*)	Elfrid ('Kormuse')	3.5 a	3.7 a	3.5 a	4.3 b	4.5 b
	Weisse Immensee ('Korweirim')	5.8 a	5.8 a	7.0 b	6.9 b	6.9 b
Total soluble sugars	Elfrid ('Kormuse')	47.7 a	63.5 c	55.1 b	66.7 c	62.1 c
	Weisse Immensee ('Korweirim')	62.3 a	76.2 b	87.3 d	82.0 c	79.8 b
Free amino acids	Elfrid ('Kormuse')	234.3 b	157.1 a	158.7 a	155.7 a	153.9 a
	Weisse Immensee ('Korweirim')	268.3 b	228.6 a	229.1 a	229.2 a	228.9 a
Polyphenolic acids	Elfrid ('Kormuse')	8.4 c	7.9 c	7.4 b	6.6 a	6.3 a
	Weisse Immensee ('Korweirim')	7.9 c	8.0 c	8.1 c	6.9 b	5.7 a

SeeTable 3 for notes.

polyphenolic acids in Elfrid ('Kormuse') by 25% and 22% and in Weisse Immensee ('Korweirim') by 28% and 13%, respectively. The use of auxin did not significantly affect the content of these compounds (Table 5).

4. Discussion

Auxins are the main group of phytohormones that are involved in rhizogenesis (de Klerk et al., 1999). IBA is one of the most effective rooting stimulators and is also applied commercially to improve the quantity and quality of ornamental plants produced from cuttings, including rose hardwood and softwood cuttings (Nasri et al., 2015; Pivetta et al., 1999). Since the effects of auxins were recognized, they have been used to stimulate rhizogenesis, especially in cuttings of the difficult-to-root species. As a consequence of the EU directives to limit the use of synthetic auxins, investigations on preparations obtained from seaweeds, citrus fruits, or crustaceans have been undertaken in an attempt to find an alternative means to stimulate plant propagation (Poşta & Hernea, 2011; Szabó & Hrotkó, 2009).

The IBA-containing preparation Rhizopon AA and the water IBA solution positively affected the degree of rooting and the percentage of rooted cuttings in deciduous leafy shrubs (Pacholczak et al., 2016). Both forms of IBA application, the powder containing 1% IBA and the foliar application of the water IBA solution (200 mg L^{-1}), improved rooting in both cultivars of ground cover roses. The best results for the rooting of plants from the family Fabaceae were obtained with the application of a powder preparation containing IBA. However, the water IBA solution provided results comparable to that of powder preparations when cuttings of *Genista tinctoria* 'Royal Gold' were rooted (Nowakowska & Pacholczak, 2015). Similarly, in the trials in this study, the effects of Rhizopon powder and the water IBA solution were comparable in regard to the degree of rooting in *Rosa* Elfrid ('Kormuse').

Recently, the use of biostimulators as an alternative to the synthetic auxin rooting enhancers has become popular. Biostimulators are generally used to improve plant growth and development (Dobrzański et al., 2008; du Jardin, 2015). In this experiment, AlgaminoPlant and Goteo were tested as alternative preparations to the commercial IBA-containing rooting stimulators. AlgaminoPlant positively affected the rooting of cuttings in the dogwoods (*Cornus alba* L.) 'Elegantissima' and 'Aurea' (Pacholczak et al., 2012). In the current experiment, the effects of biostimulators on the rooting of roses were comparable to or only slightly weaker than those of synthetic auxin IBA. These results are in agreement with those of recent trials to optimize propagation of old historical roses with such biostimulators as Bio Rhizotonic, Bio Roots, and Root Juice used to water the cuttings during the rooting period (Monder et al., 2014, 2016). Positive effects of these preparations on rooting of *Rosa gallica* 'Duchesse d'Angoulême' (Monder et al., 2014), *Rosa beggeriana* 'Polstjärnan' and *Rosa helenae* 'Semiplena' were reported (Monder & Pacholczak, 2018).

Both biostimulators in the current study, AlgaminoPlant, and Goteo, not only positively affected the degree and percentage of rooting, but also stimulated the overall growth of cuttings in both rose cultivars, especially in the new shoots of cuttings. The investigation on *Triticum aestivum* showed that the application of the preparations based on seaweeds resulted in increased root number and greater elongated new shoots (Kumar & Sahoo, 2011).

The biostimulators AlgaminoPlant and Goteo used in this study stimulated chlorophyll synthesis in the cuttings of both rose cultivars. Seaweed (*Ascophylum nodosum*) extracts applied in low concentrations increased the content of assimilation pigments in tomato leaves (Khan et al., 2009). The increase in chlorophyll levels in cuttings of two dogwood (*Cornus alba* L.) cultivars 'Aurea' and 'Elegantissima,' caused by biostimulator application, was also reported (Pacholczak et al., 2012). Conversely, a decrease in chlorophyll *a* and *b* were observed in cuttings of the historical roses *R.* ×*alba* 'Maiden Blush' and *R. helenae* 'Semiplena' treated with biostimulators (Monder et al., 2016), however rhizogenesis was not negatively affected. The change in chlorophyll concentration may be a plant response to stress, which suggests an increase in plant stress tolerance resulting from biostimulator applications (Kranner et al., 2003).

The application of IBA (Rhizopon AA or water solution) and biostimulators (Goteo and AlgaminoPlant) resulted in the increased sugar concentration in the cuttings of both cultivars. Positive effects of biostimulators and auxins on sugar levels in cuttings of historical roses have been previously reported (Monder et al., 2016; Monder & Pacholczak, 2018). Such a response in cuttings to the application of rooting enhancers is crucial for successful rhizogenesis because the effectiveness of photosynthesis in cuttings during rooting is low. New root production is a highly energetic process, such that the capability of plant material to produce carbohydrates both as storage starch and ready-to-use simple sugar forms is fundamental for rhizogenesis. The results may indicate that cuttings of ground cover roses acquired such a capability because of the application of rooting enhancers (Couvillon, 1988).

The increase in free amino acids positively affected the vitality and functioning of plant cells (Lessufleur et al., 2007). In this study, the contents of free amino acids decreased because of the treatments with all the rooting enhancers and biostimulators used. In dogwood cuttings, an increase was observed because of the application of the biopreparation AlgaminoPlant (Pacholczak et al., 2012). However, in roses treated with the biostimulators, the content of free amino acids was lower than that in the control cuttings. It could be speculated that rose cuttings treated with rooting enhancers had a more active metabolism and were intensively using free amino acid for root formation. In plants, free amino acids serve to store amine nitrogen and enable nitrogen transportation between plant organs. They are regarded as the main structural units of peptides and proteins (Costa & Spitz, 1997).

The concentration of polyphenolic acids was also decreased in cuttings of both cover roses. A similar decrease was observed in rosemary treated with 0.01% IBA (Elhaak et al., 2014). Different plants watered with solutions of Aminoplant and Goëmar Goteo also exhibited lowered levels of phenolic compounds (Rosłon et al., 2011). A different response to the treatment with rooting enhancers was observed in historical roses, where the treated cuttings contained higher or comparable amounts of polyphenolic acids as the untreated cuttings (Monder et al., 2016; Monder & Pacholczak, 2019).

5. Conclusions

The results of the experiment showed that biostimulators AlgaminoPlant and Goteo positively affected rhizogenesis in two cultivars of ground cover roses. Their effects were comparable or only slightly weaker than those of the synthetic auxin IBA. Additionally, both biopreparations stimulated the growth of new shoots in cuttings.

The use of Goteo resulted in the increased content of chlorophyll and total soluble sugars in cuttings, whereas the levels of free amino acids and polyphenolic acids were decreased. The synthetic auxin IBA increased the total sugar concentration; however, the free amino acid content was likewise decreased in the cuttings.

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