ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 23(1) 2024, 51–62

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2024.5265

ORIGINAL PAPER

Received: 30.08.20233
Accepted: 18.12.2023
Published: 28.02.2024

EFFECT OF BRASSINOSTEROIDS ON ROOTING OF THE ORNAMENTAL DECIDUOUS SHRUBS

Małgorzata Zajączkowska ✉, Andrzej Pacholczak

Section of Ornamental Plants, Faculty of Horticulture and Biotechnology, Warsaw University of Life Sciences (SGGW), Nowoursynowska 166, 02-787 Warsaw, Poland

ABSTRACT

Brassinosteroids are a developing group of growth regulators. They are a group of steroid hormones involved in plants' physiological and developmental processes. Among other things, they are responsible for cell wall regeneration or cell elongation. This experiment aimed to examine the effect of rooting stimulants on rhizogenesis in cuttings of two deciduous shrub species: *Philadelphus* 'Virginal' and *Hydrangea paniculata* 'Limelight'. Aqueous solutions of indole-3-butyric acid (IBA) at 200 mg·L^{-1}, Brassinolide (BL) at 0.05% and 24-epibrassinolide (24epiBL) (0.05%) were used in this study. The results obtained showed that both auxin and both of the brassinosteroids used increased the percentage of rooted cuttings almost twice, the degree of rooted cuttings and root length – for BL + IBA – longer roots than the control by 41% in jasmine and by 59% in hydrangea. The growth regulators applied during the rooting of cuttings also caused changes in the organic compound content of plant tissues and the activity of oxidative stress enzymes. The studies and results suggest that brassinosteroids may soon replace the popular rooting stimulants.

Key words: brassinolide, 24-epibrassinolide, indole-3-butyric acid, root system development, plant growth regulators, organic compounds

INTRODUCTION

Brassinosteroids are an increasing group of growth regulators that regulate developmental processes in ornamental plants. They are steroid compounds that were first isolated from pollen rape (*Brassica napus*), from which the name is also derived. According to literature reports, these hormones are found in all plants – at higher or lower levels [Ali 2019]. These compounds are located mainly in stem growth pods [Fridman and Savaldi-Goldstein 2013], pollen or seeds [Li et al. 2021]. In plants, they are responsible for cell division; additionally, it has been shown that they often have a crucial function in root formation and growth. However, their high concentrations can cause inhibition of differentiation and elongational growth of root meristem cells [Wei and Li 2016, Godinez-Mendoza et al. 2023]. Brassinosteroids are also characterised by a high spectrum of plant protection against biotic and abiotic stresses acting on them, such as drought, salinity and increased heavy metal content [Tanveer et al. 2018]. In addition, they are involved in shoot elongation, ethylene induction or photosynthetic enzymes [Siddiqui et al. 2018].

Numerous genes are responsible for the plant response to brassinosteroids, most of which are co-regulated by gibberellins, or primarily auxins. Genetic studies and plant physiological analyses show that the

✉ malgorzata_zajaczkowska@sggw.edu.pl

combination of this group of regulators with auxins synergises gene expression, resulting in the elongation growth of cells [Nemhauser et al. 2004]. Bao et al. [2004] confirm in their study that brassinosteroids can regulate auxin transport in the plant by accelerating it, resulting in lateral solid root development.

The brassinosteroid group includes, e.g. brassinolide (BR1), castasterone (BR2), dolicholide (BR3), teasterone (BR8), 28-norbrassinolide (BR14), 28-homobrassinolide (BR17), 24-epibrassinolide (BR27), secasterone (BR38), 28-nortyphasterol (BR49), secasterol (BR53) [Baqer et al. 2019]. The most common rooting preparations are 3 of these, brassinolide, 24-epibrassinolide and 28-homobrassinolide, which are applied as a spray with a water solution or as an additive to media *in vitro* [Gomes 2011].

To demonstrate the relevance of the use of this group of growth regulators in the vegetative propagation of ornamental deciduous shrubs, this article presents several aspects of the effects on the development of the root system, as well as on the changes occurring in plant tissues in terms of organic substances, using the example of selected shrub species: hydrangea and jasmine.

MATERIAL AND METHODS

The research was carried out over two growing seasons in 2021 and 2022. The research used one-year-old one-node shoot cuttings obtained from 7-year-old shrubs: *Philadelphus* (jasmine) 'Virginal' and *Hydrangea paniculata* (bouquet hydrangea) 'Limelight', which were placed in polystyrene boxes filled with a mixture of peat, coconut fibre and perlite at a ratio of 2:1:1 and a pH of 6.0. Aqueous solutions of 3 growth regulators: indole-3-butyric acid (IBA) at a concentration of 200 mg·L^{-1}, a 0.05% BL solution or 24epiBL (Sigma Aldrich®, St. Louis, MO, USA), which was pre-dissolved in 95% ethanol, or a combination of brassinosteroids and IBA auxin, were used as stimulators of root system production in the form of a single spray at the time the cuttings were placed in the medium (Fig. 1). The concentrations used in the experiment were chosen based on previous preliminary studies and available literature sources.

After all the combinations had been applied, the polystyrene containers and the cuttings were placed in plastic tunnels where a spraying and shading system was installed. The temperature during rooting ranged from 25–31°C during the day to 15–20°C at night.

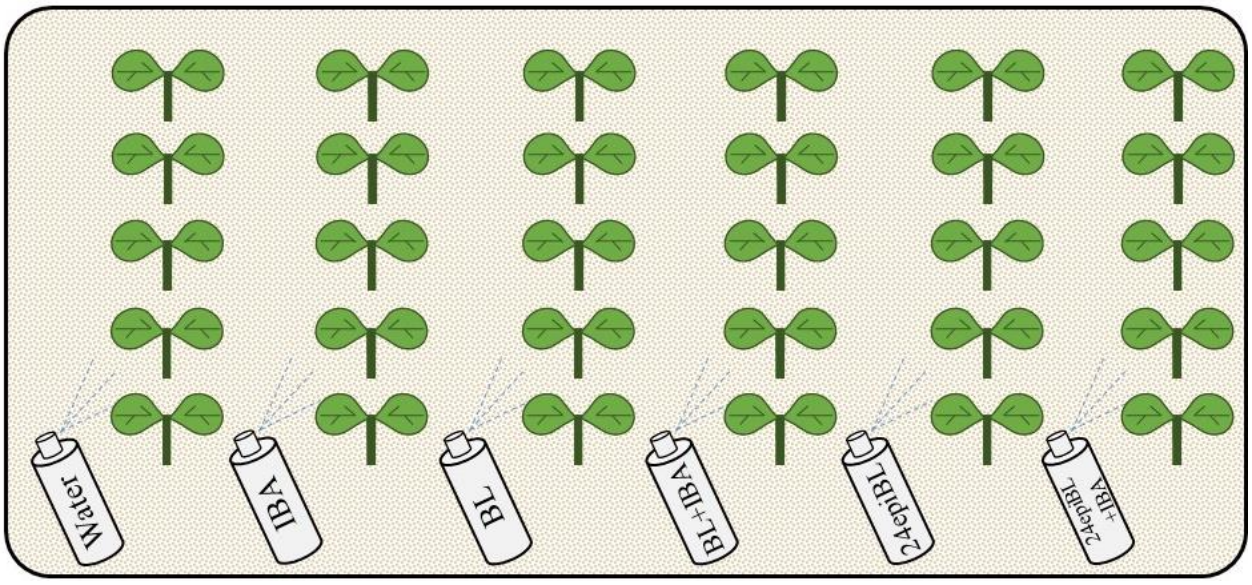

Fig. 1. Scheme of the experiment – cuttings placed in the substrate sprayed with (from left) control – distilled water, IBA – 200 mg·L^{-1}, BL – 0.05%, BL – 0.05% + IBA – 200 mg·L^{-1}, 24epiBL – 0.05% and 24epiBL 0.05% + IBA – 200 mg·L^{-1}

After seven weeks, the percentage and degree of rooted cuttings were scored. For this purpose, a 5-grade rating scale was determined to define an adequately developed root ball (Tab. 1).

In addition, several biochemical analyses were carried out to investigate the effect of the growth regulators used on changes in the content of organic compounds. All biochemical analyses were performed in triplicate. Samples of leaf blades were stored at –84°C until the analyses were carried out.

Chlorophyll and carotenoid content – plant material (0.5 g) was ground in a mortar with quartz sand and 5 mL of 80% cold acetone. The extracts obtained were then filtered through filter paper into 50 mL volumetric flasks and filled up to the mark with acetone. After obtaining clear filtrates, the absorbance was measured spectrophotometrically with the spectrophotometer UV-1601 PC (Shimadzu, Columbia, MD, USA) at four wavelengths – 470, 646, 652 and 663 nm [Lichtenthaler and Wellburn 1983].

Total sugars content – 0.5 g of sample (3 of each species) was ground with a mortar in hot 80% ethanol. It was then centrifuged in a centrifuge for 20 minutes at 20,000 rpm at 4°C. After centrifugation, the supernatant had to be transferred into resealable tubes and supplemented to 25 mL with ethanol (80%). For analysis, 100 µl of the extract was taken, 1 mL of 5% phenol was added, and 5 mL of sulphuric acid (H_2SO_4, 96%) was added. After mixing, the solutions were allowed to incubate for 20 minutes. After the time had elapsed, the absorbance was measured against a standard curve for glucose at 490 nm [Dubois et al. 1956].

Free amino acid content – 0.2 mL of the supernatant obtained from grinding the plant material for sugar analysis – was taken into resealable measuring tubes, and 0.8 mL of ethanol (80%) was added. Then 0.5 mL of 0.2 mM sodium cyanide in acetate buffer, pH 5.3–5.4, and 0.5 mL of ninhydrin were added. The prepared solutions were mixed and incubated in a water bath (100°C) for 15 minutes. After this, the tubes were removed, and 5 mL of isopropyl alcohol was added to the warm tubes. After pouring, the mixtures were allowed to cool. Once thoroughly cooled, the absorbance was measured at 570 nm against a standard curve for leucine [Rosen 1957].

Soluble protein content – 0.1 mL of each supernatant obtained from grinding the plant material for the sugar analysis was taken into tubes, and 5 mL of Bradford reagent was added. After thorough mixing, it was allowed to incubate for 5 minutes at room temperature. After the time had elapsed, absorbance was measured at 595 nm [Bradford 1976].

Hydrogen peroxide (H_2O_2) – samples of plant material (from each species and each combination of three samples) were ground in K-phosphate buffer using a mortar. After mashing and pouring into plastic tubes, they were centrifuged for 20 min at 20,000 rpm at 4°C. The supernatants obtained were collected in glass tubes, from which the extract was taken for further steps. 0.1 mL of each extract was taken into glass tubes and made up to 0.5 mL with K-phosphate buffer. To the resulting solution, 0.5 mL of 0.1 M K-phosphate buffer and 1 mL of 1 M potassium iodide (KI) had to be added. The mixture was mixed and incubated in the dark for one hour. When the time had elapsed, the absorbance was measured at 390 nm [Siedlecka 2010].

Catalase activity (CAT) – supernatants obtained during the H_2O_2 determination were used for the analysis. The tubes had to be divided into two groups, of which 0.05 mL of each extract was taken and then supplemented with 0.45 mL K-phosphate buffer. To the

Table 1. Evaluation of root system development

Degree of rooting of the cuttings	Score
Unrooted cuttings	1
1–2 single roots without visible root ball	2
3–4 slightly branched roots and medium-sized root ball	3
6–10 branched roots and well-developed root ball	4
>10 well-branched roots, well-developed root ball	5

group of tubes marked 'A', 1 mL of 0.1 M K-phosphate buffer was added, and to the other group 'B', 1 mL of H_2O_2 in buffer (65 μM) was added. Sample 'K' (control) was still prepared; 1.5 mL of 0.1 M K-phosphate buffer was poured in, and sample 'C', where 0.5 mL of 0.1 M K-phosphate buffer was mixed with 1 mL of H_2O_2 in buffer (65 μM). All tubes had to be incubated in the dark for 10 min. After this time, 1 mL of 32.5 mM ammonium molybdate was to be added to all of them and mixed. After thorough mixing, absorbance was measured at 405 nm [Góth 1991].

All collected results were statistically analysed using Statgraphics Centurion XVI software (Statgraphics Technologies, Inc., The Plains, VA, USA). For all parameters, a one-way analysis of variance was performed, and Tukey's multiple comparisons test was used to group the means at a significance level of $\alpha = 0.05$.

RESULTS

Brassinosteroids and root system development. Studies conducted by the authors show that using both the brassinosteroids–BL and 24epiBL alone and in combination with auxin IBA results in improved rooting of cuttings. The results presented in Table 2 show that the regulation of rhizogenesis depends mainly on the species. The highest results for the percentage of rooted cuttings were obtained in the case of hydrangea 'Limelight' in 2022, as the three combinations where plants were treated with BL, BL + IBA, and 24epiBL resulted in 70% and higher. The number of rooted cuttings between the different combinations differed slightly in the second species – jasmine. However, compared to the control, the values were higher by up to 36.6% concerning the control.

The results obtained for the two deciduous shrub species tested show that the interaction of brassinosteroids with auxin IBA, but mainly BL and 24epiBL alone, improves root ball development, where this was confirmed in both years of the study (Tab. 3 and Fig. 2). There was a noticeable difference in the 2022 hydrangea cuttings in the combination where 24epiBL was applied, as the degree of rooting increased by 20% compared to the control.

The length of individual roots is also an essential parameter in the structure of the root system. The experiment proved that the use of brassinosteroid growth regulators as rooting stimulants resulted in faster root growth in length (Tab. 4 and Fig. 3). The longest roots in both 2021 and 2022 were characterised by BL + IBA-treated cuttings in jasmine, with approximately 41% longer roots than in control, and in hydrangea BL + IBA and 24epiBL, with approximately 59% longer root systems in cuttings of both combinations relative to the others. However, all cuttings from the brassinosteroid-treated combinations had longer roots than the control cuttings.

Biochemical changes in cuttings under brassinosteroid treatments. In a study conducted on two deciduous shrub species, additional treatment of cuttings with brassinosteroids was found to increase chlorophyll content, but this depended on the species. For jasmine, the value increased only in the combination where 24epiBL was used – by more than 12% in 2022 compared to the control. Hydrangea cuttings responded better to the brassinosteroid treatment, as, relative to untreated cuttings, the highest contents were observed in the combinations using BL and 24epiBL (10% and 7% more) in 2022. Furthermore, applying IBA and its combination with BL in 2022 increased young hydrangea plants' chlorophyll content by 6% (Tab. 5).

The highest content of carotenoids in both years of the study was observed in the combination where the cuttings were treated with 24epiBL, in jasmine by 48%, and in hydrangea by 12% more compared to the control (2022). For the second plant, carotenoid content increased in the BL and BL + IBA combinations in 2022 – above 2 $mg \cdot g^{-1}$ d.w. (Tab. 5).

The study conducted on the two deciduous shrub species showed that the applied growth regulators worked better in hydrangea (Tab. 6). An increase in total sugars in jasmine in 2022 was noticed in the combinations 24epiBL + IBA – by 31% and 24epiBL – by 38% more than the control. The same parameter tested in hydrangea in 2022 gave much more effective results, as all combinations, except BL, showed a significant increase in sugar concentration. However, the highest result was observed in material taken from cuttings treated with 24epiBL, which was 39% higher than the control (2022). Besides, the content of this compound in hydrangea compared to jasmine was higher by up to 13 times – for the 24epiBL combination.

Table 2. Percentage of rooted cuttings after brassinosteroids and IBA treatments

Species	Year	Control	IBA	BL	BL + IBA	24epiBL	24epiBL + IBA
Philadelphus 'Virginal'	2021	38.3 ±4.7 a	55.0 ±8,2 ab	46.7 ±6.2 ab	63.3 ±6.2 b	56.7 ±8.5 ab	56.7 ±2.4 ab
	2022	43.3 ±6.2 a	58.3 ±2.4 bc	51.7 ±2.4 ab	68.3 ±2.4 c	58.3 ±6.2 bc	60.0 ±4.1 bc
Hydrangea paniculata 'Limelight'	2021	38.3 ±4.7 a	46.7 ±4.7 ab	63.3 ±6.2 bc	60.0 ±7.1 bc	68.3 ±6.2 c	48.3 ±2.4 ab
	2022	41.7 ±8.2 a	63.3 ±6.2 b	70.0 ±7.1 b	70.0 ±0.0 b	76.7 ±9.4 b	63.3 ±6.2 b

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation

Table 3. Effect of brassinosteroids and auxin IBA on the degree of rooting of cuttings

Species	Year	Control	IBA	BL	BL + IBA	24epiBL	24epiBL + IBA
Philadelphus 'Virginal'	2021	2.1 ±0.1 a	2.2 ±0.1 ab	2.5 ±0.1 bc	2.6 ±0.0 c	2.5 ±0.2 bc	2.6 ±0.1 c
	2022	2.1 ±0.1 a	2.2 ±0.1 ab	2.6 ±0.1 c	2.5 ±0.2 bc	2.6 ±0.1 c	2.5 ±0.2 bc
Hydrangea paniculata 'Limelight'	2021	2.2 ±0.0 a	2.5 ±0.1 ab	2.6 ±0.3 ab	2.6 ±0.2 ab	2.9 ±0.1 b	2.8 ±0.1 b
	2022	2.4 ±0.2 a	2.8 ±0.2 b	2.9 ±0.3 b	2.8 ±0.1 b	3.0 ±0.1 b	3.0 ±0.1 b

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation

Table 4. Effect of brassinosteroids and auxin IBA on the root length in cuttings

Species	Year	Control	IBA	BL	BL + IBA	24epiBL	24epiBL + IBA
Philadelphus 'Virginal'	2021	1.5 ±0.1 a	2.5 ±0.1 c	2.1 ±0.0 b	2.6 ±0.1 c	2.2 ±0.0 b	2.1 ±0.0 b
	2022	1.6 ±0.1 a	2.6 ±0.2 cd	2.2 ±0.2 bcd	2.7 ±0.1 d	2.2 ±0.0 bc	2.1 ±0.1 b
Hydrangea paniculata 'Limelight'	2021	1.8 ±0.1 a	2.6 ±0.3 b	3.2 ±0.2 c	3.8 ±0.0 d	3.8 ±0.1 d	3.2 ±0.1 c
	2022	1.6 ±0.2 a	2.8 ±0.2 b	3.4 ±0.3 c	3.9 ±0.1 d	3.9 ±0.1 d	3.4 ±0.2 c

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation

Fig. 2. Effect of brassinosteroids and IBA on root length and degree of rooting in *Philadelphus* 'Virginal' cuttings. From left: 1 – control, 2 – IBA, 3 – BL, 4 – BL + IBA, 5 – 24epiBL and 6 – 24epiBL + IBA

The research showed that in 2022, in jasmine, the only combination that increased free amino acid concentration was the one where the cuttings were treated with BL, but this was higher than the control by only 5% (Tab. 6). A similar situation, where BL but also BL + IBA caused an increase in the content of these compounds, was observed in hydrangea in both years of the study; for BL, it increased by 4% relative to the control, while for BL + IBA, it increased by 8% (year 2022).

It is interesting to note that although jasmine cuttings in all combinations rooted very well, the protein content regarding the control was lower in both years of the experiments, in some cases decreasing in content by up to 3 times (24epiBL + IBA – 2022). In contrast, the opposite effect of the growth regulators was observed in hydrangea, which caused an increase in protein content in both years. The highest result was in the combination where cuttings were treated with BL with IBA – over 58% more than in the control in 2021 and 2022 (Tab. 6).

In jasmine, due to the low H_2O_2 in all combinations using growth regulators, CAT activity was at a lower level concerning the material taken from control cuttings in both years of the study. However, there was an increase in H_2O_2 concentration in hydrangea, except in the combination where the cuttings were sprayed with BL + IBA – 22% less relative to the control – year 2022. In the same year for this combination, as well as in those using BL alone and 24epiBL, CAT activity increased significantly relative to the control. Only in the combinations of IBA (2022) and its combination with 24epiBL (2022) was there no change in the activity of this oxidative stress enzyme (Tab. 7).

DISCUSSION

Brassinosteroids and root system development

A well-developed and healthy root system plays a vital role in the proper development and growth of plants. It is primarily responsible for holding the plant

Table 5. Effect of brassinosteroids on changes in plant pigment content

Parameter	Species	Year	Control	IBA	BL	BL + IBA	24epiBL	24epiBL + IBA
chlorophyll [mg·g^{-1} d.w.]	*Philadelphus* 'Virginal'	2021	2.02 ±0.01 a	1.90 ±0.09 a	1.82 ±0.04 a	2.06 ±0.03 ab	2.27 ±0.03 bc	2.40 ±0.13 c
		2022	2.77 ±0.06 c	2.43 ±0.01 a	2.57 ±0.01 b	2.54 ±0.05 b	3.16 ±0.06 d	2.38 ±0.01 a
carotenoids [mg·g^{-1} d.w.]		2021	0.23 ±0.00 b	0.21 ±0.00 a	0.20 ±0.01 a	0.21 ±0.00 a	0.26 ±0.00 c	0.20 ±0.01 a
		2022	0.54 ±0.02 b	0.73 ±0.01 c	0.51 ±0.02 b	0.32 ±0.01 a	1.04 ±0.02 d	0.61 ±0.01 bc
chlorophyll [mg·g^{-1} d.w.]	*Hydrangea paniculata* 'Limelight'	2021	3.77 ±0.00 b	3.69 ±0.03 a	4.74 ±0.01 f	4.00 ±0.01 e	4.21 ±0.01 d	3.92 ±0.02 c
		2022	8.42 ±0.01 a	8.89 ±0.03 b	9.31 ±0.03 d	8.98 ±0.03 b	9.08 ±0.04 c	8.41 ±0.03 a
carotenoids [mg·g^{-1} d.w.]		2021	1.82 ±0.01 b	1.68 ±0.01 a	1.66 ±0.05 a	1.68 ±0.01 a	2.07 ±0.01 c	1.60 ±0.06 a
		2022	1.90 ±0.03 a	1.94 ±0.02 a	2.01 ±0.02 b	2.03 ±0.01 b	2.15 ±0.03 c	1.93 ±0.00 a

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation

Table 6. Effect of brassinosteroids on organic compound content in the species studied

Parameter	Species	Year	Control	IBA	BL	BL + IBA	24epiBL	24epiBL + IBA
total soluble sugar [mg·g^{-1} d.w.]	*Philadelphus* 'Virginal'	2021	22.53 ±0.14 b	22.65 ±0.22 b	30.59 ±0.04 c	29.71 ±0.53 c	21.19 ±0.65 a	21.03 ±0.42 a
		2022	14.93 ±1.92 a	13.45 ±1.23 a	13.42 ±0.61 a	15.96 ±0.61 a	24.18 ±1.71 b	21.72 ±2.26 b
free amino acids [µmol leucine g^{-1} d.w.]		2021	220.23 ±2.62 c	206.09 ±1.84 b	210.07 ±0.62 b	207.92 ±1.57 b	179.83 ±4.31 a	182.55 ±0.85 a
		2022	272.62 ±5.66 d	256.73 ±1.71 b	285.50 ±3.01 e	250.00 ±1.61 b	264.14 ±1.68 c	176.96 ±3.09 a
soluble protein [mg·g^{-1} d.w.]		2021	3.97 ±0.01 f	2.88 ±0.03 e	2.74 ±0.01 d	2.32 ±0.01 b	2.41 ±0.01 c	1.87 ±0.01 a
		2022	4.55 ±0.01 f	3.79 ±0.01 e	3.39 ±0.01 d	1.66 ±0.01 b	1.97 ±0.01 c	1.48 ±0.02 a
total soluble sugar [mg·g^{-1} d.w.]	*Hydrangea paniculata* 'Limelight'	2021	194.14 ±0.13 a	235.47 ±0.23 c	198.11 ±0.03 b	285.71 ±0.46 d	326.63 ±0.55 e	285.89 ±0.36 d
		2022	191.52 ±2.04 b	229.30 ±1.41 cd	175,49 ±2.98 a	239.27 ±1.51 d	312.50 ±2.70 e	215.81 ±2.30 c
free amino acids [µmol leucine g^{-1} d.w.]		2021	139.75 ±1.28 a	146.81 ±0.55 b	189.15 ±0.18 d	202.06 ±1.23 e	152.05 ±0.76 c	141.58 ±0,46 a
		2022	155.70 ±0.64 b	157.64 ±0.94 b	161.42 ±0.64 c	168.32 ±0.65 d	155.45 ±0.99 b	128.42 ±0.68 a
soluble protein [mg·g^{-1} d.w.]		2021	0.56 ±0.00 a	0.72 ±0.00 b	1.35 ±0.00 e	1.47 ±0.00 f	1.15 ±0.00 d	1.09 ±0.00 c
		2022	0.77 ±0.00 a	0.91 ±0.04 b	1.63 ±0.03 d	1.76 ±0.04 e	1.48 ±0.05 c	1.47 ±0.03 c

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation

Table 7. Effects of brassinosteroids on parameters corresponding to the plant stress response

Parameter	Species	Year	Control	IBA	BL	BL + IBA	24epiBL	24epiBL + IBA
H_2O_2 [µg·g^{-1} d.w.]	*Philadelphus* 'Virginal'	2021	185.31 ±4.07 c	149.31 ±1.52 a	152.08 ±4.48 a	167.85 ±3.63 b	143.13 ±0.82 a	147.75 ±0.98 a
		2022	175.52 ±0.51 d	151.99 ±0.66 c	108.23 ±0.85 a	107.93 ±0.70 a	128.78 ±0.74 b	129.87 ±0.86 b
catalase [U·g^{-1} d.w.]		2021	2302.50 ±20.66 d	865.08 ±57.71 a	1235.48 ±64.39 b	1750.81 ±100.33 c	1647.47 ±69.37 c	2190.56 ±49.57 d
		2022	4418.51 ±117.67 e	1598.77 ±106.65 a	2121.14 ±110.55 b	3028.30 ±173.54 c	2964.58 ±124.83 c	3850.90 ±87.14 d
H_2O_2 [µg·g^{-1} d.w.]	*Hydrangea paniculata* 'Limelight'	2021	130.76 ±1.21 b	193.70 ±0.46 e	209.68 ±1.28 f	124.53 ±1.05 a	149.81 ±0.24 c	160.93 ±0.29 d
		2022	165.95 ±0.80 b	209.52 ±1.49 d	221.62 ±0.47 e	128.83 ±0.69 a	171.78 ±0.64 b	189.30 ±0.27 c
catalase [U·g^{-1} d.w.]		2021	5919.12 ±53.12 d	2255.97 ±150.49 a	3050.41 ±158.98 b	4390.38 ±251.60 c	4139.45 ±174.30 c	5596.77 ±126.64 d
		2022	6299.80 ±5.45 a	6308.22 ±2.42 a	6673.95 ±1.87 b	7970.83 ±0.69 c	8631.94 ±0.80 d	6179.05 ±0.78 a

* the same letter in the lines indicates no difference between the means at a significance level of $\alpha = 0.05$ ± means standard deviation

in the substrate, providing the necessary mineral substances with water, and synthesising plant hormones that promote root development. Auxins and brassinosteroids show such effects [Lv et al. 2018]. They are the ones that, at low concentrations, stimulate the cells of the root apical meristem to divide and elongate the growth of cuttings [Brosa 1999].

Using brassinosteroids while rooting both hydrangea and jasmine showed that the percentage of rooted cuttings increased regarding control cuttings. Experiments have already been conducted on coniferous plants in which treatment with brassinosteroids during the rooting process of spruce – *Picea abies* (L.) – cuttings had positive effects by accelerating the initiation of root system development [Rönsch et al. 1993]. Pacholczak et al. [2021] obtained similar results for barberry (*Berberis thunbergia* 'Maria' and 'Red Rocket') cuttings. The applied brassinosteroids and the combination with IBA resulted in an improved percentage of rooted cuttings. In addition, the degree of rooting of cuttings obtained the highest results in combinations using brassinosteroids with IBA. Regarding the experiment carried out by the authors, confirmation was obtained that using brassinosteroids improves the root system's structure in ornamental shrubs.

The length of the root system can also contribute to the proper growth and development of cuttings. The authors' work noted the critical role of the growth regulators, particularly the high importance of brassinosteroids. In both hydrangea and jasmine, root length increased relative to the length measured in control cuttings; however, the highest average lengths were those in which regulators from the brassinosteroid group were applied. Current studies on the effects of this group of plant hormones indicate that they have a significant effect on the length of the root system, as reported in cucumber (*Cucumis sativus* L.) cuttings after application of 24epiBL – an increase in root length of more than 66% compared to the control [Xia et al. 2009] and in apple (*Malus hupehensis* Rehd.) cuttings treated with BL – a twice more extended root system [Mao et al. 2017] were noted.

Biochemical changes in cuttings under brassinosteroid treatments Compounds in plant tissues, especially plant pigments [Khaleghi et al. 2012], sugars [Husen 2012] or proteins [Han et al. 2009], play an essential role in successful root and shoot/leaf growth. Studies have strongly suggested that the content of individual organic compounds depends on the species. In addition, applying growth regulators produced different effects depending on the genotype studied.

Plant pigments play a crucial role in the proper functioning of plants. Chlorophyll is responsible for absorbing light, converting it into the energy needed for proper photosynthesis [Khaleghi et al. 2012]. The concentration of this plant pigment largely depends on the species and, above all, the cultivar, so the levels measured in plant tissues are different. Moreover, many changes in content are caused by external factors occurring during growth or rooting [Fini et al. 2010, Tribulato et al. 2019]. During a two-year study, we observed that using brassinosteroids as rooting stimulants and improving rooting efficiency increases the essential plant pigments such as chlorophyll. Spraying with 24epiBL increased the chlorophyll content in cucumber plants [Xia et al. 2009]. A positive effect of brassinosteroid application on chlorophyll content was also shown in cucumber cuttings studied by Yu et al. [2004] and Indian nickel *Cajanus cajan* (L.) Millsp. [Dalio et al. 2011].

Other pigments contained in the plant are carotenoids, which respond to oxidative stress affecting plants. Their increased accumulation protects plant cells against photo-oxidative damage and heat stress [Rosas-Saavedra and Stange 2016]. In the experiment, it was observed that the application of brassinosteroids allowed an increase in the carotenoid content of plant tissues; however, the levels in the different combinations varied according to the species treated. For example, in jasmine, the level of carotenoids was the highest in combination treated by IBA, 24epiBL and 24epiBL + IBA, but in hydrangea, in every combination level of these pigments was lower than the control. Changes in the level of carotenoids were correlated with treated species. Moreover, such a relationship was already apparent in the available literature, even at the variety stage. Studies on two flax varieties (*Linum usitatissimum* L.) treated with brassinosteroids showed that one variety responded by increasing carotenoid content in seedlings, while the other responded by decreasing it [Amraee et al. 2020]. Applying different concentrations of 24epiBL in serpentine horsegram seeds (*Vigna unguiculata* L. Walp.) in-

creased the concentration of these plant pigments [Lima and Lobato 2017].

Another critical aspect of plant tissue structure is sugars, responsible for enzymatic regulation. They influence the regulation of many growth processes, cell differentiation, vegetative plant growth or flowering processes. These compounds act as building materials in plant cells and have structural or protective functions [Husen 2012]. Our experiment confirmed the beneficial effect of brassinosteroids on the content of the total sugars. It was observed that a practical effect, in particular, fell for the combination using 24epiBL, both in hydrangea and jasmine. Similar results were obtained by Prakash et al. [2003], who found brassinosteroids to be responsible for the increase in sugar levels in *Arachis hypogaea* L. Pacholczak et al. [2021] in their study presented an identical effect. They observed an increase in sugar levels of barberry 'Maria' and 'Red Rocket' varieties. Also, cucumber plants treated with 24-epiBL had higher sugar levels than the others [Xia et al. 2009].

Free amino acids are other essential compounds responsible for plant tissue regeneration under stress factors [Vardhini 2014]. They accumulate in plants and are crucial in regulating ion transport, opening stomatal apparatuses, or detoxifying heavy metals. In addition, they influence the synthesis and activity of certain enzymes and gene expression [Rai 2002]. In the jasmine and hydrangea, brassinosteroids, particularly BL, were observed to improve the accumulation of free amino acids. Amraee et al. [2020] found in their study that this group of compounds, the brassinosteroids, can beneficially affect plant tissues' free amino acid content. Androgen binding proteins (ABP), which were found, among others, in the cell membrane, were auxin receptors and, as a result, proteins in plants have had an essential effect on rhizogenesis [Effendi and Scherer 2011]. Proteins play a significant role in the reconstruction of missing tissues. Their content largely determines the success of the rooting process of cuttings [Han et al. 2009]. Our two-year study has shown the varied effects of brassinosteroids on the content of proteins. The plant material analysed showed that the effect of this group of growth regulators strongly correlates with the species to which it is applied. For example, in jasmine, the highest level was observed in the combination without any growth regulators, but in hydrangea, the effects were different because the level of soluble protein was higher than the control in all the treatments. In the literature, differences are also observed at the cultivar level. For example, an experiment conducted by Amraee et al. [2020] on two varieties of *L. usitatissimum* found that the application of 24epiBL caused a decrease in protein concentration in one of the varieties (TN-97-1), while in the other, it was maintained at a comparable level to the control. A study on rice (*Oryza sativa* L.) seedlings showed that the soluble protein content increased compared to control plants after applying brassinosteroid compounds [Anuradha and Rao 2001].

The balance of compounds produced during stress factors is essential in adequately rooting cuttings. One of these is H_2O_2, which adversely affects metabolic processes in the plant [Bajguz and Hayat 2009]. The typical CAT reaction involves the dismutation of an H_2O_2 molecule to water and oxygen. Cellular organelles exhibiting this enzyme's high activity are peroxisomes [Mhamdi et al. 2010]. In their study, the authors observed that the H_2O_2 content and the correlated CAT activity were species-dependent. The application of growth regulators in the rhizogenesis process in jasmine had a positive effect, as the H_2O_2 content was decreased in our experiment. However, a variable effect of brassinosteroid application on H_2O_2 accumulation and CAT activity was noted in hydrangea. The results of Behnamnia et al. [2009] show that higher brassinolide concentrations strongly reduce H_2O_2 levels. Zhu et al. [2016] observed that applying a brassinosteroid growth hormone improved CAT synthesis in tomato cuttings.

CONCLUSIONS

Studies carried out by the authors of this paper demonstrate that the application of hormones from the brassinosteroid group (brassinolide and 24-epibrassinolide) improves the rooting quality of cuttings – jasmine (*Philadelphus*) 'Virginal' and bouquet hydrangea (*Hydrangea paniculata*) 'Limelight'. In addition, many biochemical studies confirmed these regulators' ability to modify the content of specific plant tissue-building compounds and the activity of stress enzymes. It is due to external conditions, particularly in cuttings during rooting, which are exposed to water deficit or temperature fluctuations.

It should be noted that the experiments and analy-

ses carried out provide a basis for further analysis of the interaction of brassinosteroids with other growth regulators, such as the auxin IBA used in the study. In addition, it becomes crucial to understand the changes occurring in plant tissue structures.

THE SOURCE OF FUNDING

Władysław Grabski's Main Library, Warsaw University of Life Sciences, Warsaw, Poland.

REFERENCES

Ali, B. (2019). Brassinosteroids: The promising plant growth regulators in horticulture. In: Brassinosteroids: plant growth and development, Hayat, S., Yusuf, M., Bhardwaj, R., Bajguz, A. (eds.). Springer, Singapore, 349–365. https://doi.org/10.1007/978-981-13-6058-9_12

Amraee, L., Rahmani, F., Mandoulakani, B.A. (2020). Exogenous application of 24-epibrassinosteroid mitigates NaCl toxicity in flax by modifying free amino acids profile and antioxidant defence system. Funct. Plant Biol. 47(6), 565–575. https://doi.org/10.1071/fp19191

Anuradha, S., Rao, S.S.R. (2001). Effect of brassinosteroids on salinity stress induced inhibition of seed germination and seedling growth of rice (*Oryza sativa* L.). Plant Growth Reg. 33, 151–153. https://doi.org/10.1023/A:1017590108484

Bajguz, A., Hayat, S. (2009). Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiol. Biochem. 47(1), 1–8. https://doi.org/10.1016/j.plaphy.2008.10.002

Bao, F., Shen, J., Brady, S.R., Muday, G.M., Asami, T., Yang, Z. (2004). Brassinosteroids interact with auxin to promote lateral root development in *Arabidopsis*. Plant Physiol. 134(4), 1624–1631. https://doi.org/10.1104/pp.103.036897

Baqer, H.A., Al-Hassan, M.F.H., Mahmod, J.W. (2019). Role of brassinosteroids (BRs) in plants. J. Res. Ecol. 7(2), 2555–2563.

Behnamnia, M., Kalantari, K.M., Ziale, J. (2009). The effects of brassinosteroids on the induction of biochemical changes in *Lycopersicon esculentum* under drought stress. Turk. J. Biol. 33(6), 417–428. https://doi.org/10.3906/bot-0806-12

Bradford, M.M. (1976). A rapid and sensitive metod for the quantification of microgram quantities of protein utilizng the principle of protein dye binding. Anal. Biochem. 72(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Brosa, C. (1999). Biological effects of brassinosteroids. Crit. Rev. Biochem. Mol. Biol. 34(5), 339–358. https://doi.org/10.1080/10409239991209345

Dalio, R.J.D., Pinheiro, H.P., Sodek, L., Baptista Haddad, C.R. (2011). The effect of 24-epibrassinolide and clotrimazole on the adaptation of *Cajanus cajan* (L.) Millsp. to salinity. Acta Physiol. Plant. 33, 1887–1896. https://doi.org/10.1007/s11738-011-0732-x

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. (1956). Colometric method for determination of sugars and related substances. Anal. Chem. 28(3), 350–356. https://doi.org/10.1021/ac60111a017

Effendi, Y., Scherer, G.F.E. (2011). Auxin binding-protein1 (ABP1), a receptor to regulate auxin transport and early auxin genes in an interlocking system with PIN proteins and the receptor TIR1. Plant Signal. Behav. 6(8), 1101–1103. https://doi.org/10.4161/psb.6.8.16403

Fini, A., Ferrini, F., Frangi, P., Piatti, R., Amoroso, G. (2010). Effects of shading on growth, leaf gas exchange and chlorophyll fluorescence of three container-grown shrubs. Acta Hortic. 885, 109–117. https://doi.org/10.17660/actahortic.2010.885.14

Fridman, Y., Savaldi-Goldstein, S. (2013). Brassinosteroids in growth control: How, when and where. Plant Sci. 209, 24–31. https://doi.org/10.1016/j.plantsci.2013.04.002

Godínez-Mendoza, P.L., Rico-Chávez, A.K., Ferrusquía-Jimenez, N.I., Carbajal-Valenzuela, I.A., Villagómez-Aranda, A.L., Torres-Pacheco, I., Guevara-González, R.G. (2023). Plant hormesis: Revising of the concepts of biostimulation, elicitation and their application in a sustainable agricultural production. Sci. Total Environ. 894, 164883. https://doi.org/10.1016/j.scitotenv.2023.164883

Gomes, M.M.A. (2011). Physiological effects related to brassinosteroid application in plants. In: Brassinosteroids: a class of plant hormone. Hayat, S., Ahmad, A. (eds.), Springer, Dordrecht, 193–242. https://doi.org/10.1007/978-94-007-0189-2_7

Góth, L. (1991). A simple method for determination of serum catalase activity and revision range. Clin. Chim. Acta 196(2–3), 143–152. https://doi.org/10.1016/0009-8981(91)90067-m

Han, H., Zhang, S., Sun, X. (2009). A review on the molecular mechanism of plants rooting modulated by auxin. Afr. J. Biotechnol. 8(3), 348–353.

Husen, A. (2012). Changes of soluble sugars and enzymatic activities during adventitious rooting in cuttings of *Grewia optiva* as affected by age of donor plants and auxin treatments. Am. J. Plant Physiol. 7(1), 1–16. https://doi.org/10.3923/ajpp.2012.1.16

Khaleghi, E., Arzani, K., Moallemi, N., Barzegar, M. (2012). Evaluation of chlorophyll content and chlorophyll fluorescence parametrs and relationships between

chlorophyll a, b and chlorophyll content index under water stress in *Olea europaea* cv. Dezful. World Acad. Sci. Eng. Technol. 6(8), 1154–1157.

Li, S., Zheng, H., Lin, L., Wang, F., Sui, N. (2021). Roles of brassinosteroids in plant growth and abiotic stress response. Plant Growth Regul. 93(1), 29–38. https://doi.org/10.1007/s10725-020-00672-7

Lichtenthaler, H.K., Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b leaf extracts in different solvents. Biochem. Soc. Trans. 11(5), 591–592. https://doi.org/10.1042/bst0110591

Lima, J.V., Lobato, A.K.S. (2017). Brassinosteroids improve photosystem II efficiency, gas exchange, antioxidant enzymes and growth of cowpea plants exposed to water deficit. Physiol. Mol. Biol. Plants 23(1), 59–72. https://doi.org/10.1007/s12298-016-0410-y

Lv, B., Tian, H., Zhang, F., Liu, J., Lu, S., Bai, M., Li, C., Ding, Z. (2018). Brassinosteroids regulate root growth by controlling reactive oxygen species homeostasis and dual effect on ethylene synthesis in *Arabidopsis*. PLoS Gen. 14(1), e1007144. https://doi.org/10.1371/journal.pgen.1007144

Mao, J., Zhang, D., Li, K., Liu, Z., Liu, X., Song, C., Li, G., Zhao, C., Ma, J., Han, M. (2017). Effect of exogenous Brassinolide (BR) application on the morphology, hormone status, and gene expression of developing lateral roots in *Malus hupehensis*. Plant Growth Reg. 82, 391–401. https://doi.org/10.1007/s10725-017-0264-5

Mhamdi, A., Queval, G., Chaouch, S., Vanderauwera, S., Van Breusegem, F., Noctor, G. (2010). Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models. J. Exp. Bot. 61(15), 4197–4220. https://doi.org/10.1093/jxb/erq282

Nemhauser, J.L., Mockler, T.C., Chory, J. (2004). Interdependency of brassinosteroids and auxin signaling in Arabidopsis. PLoS Biol. 2(9), 1460–1471. https://doi.org/10.1371/journal.pbio.0020258

Pacholczak, A., Zajączkowska, M., Nowakowska, K. (2021). The effect of brassinosteroids of rooting of stem cuttings in two Barberry (*Berberis thunbergii* L.) cultivars. Agronomy 11(4), 699. https://doi.org/10.3390/agronomy11040699

Prakash, M., Saravanan, K., Sunil Kumar, B., Ganesan, J. (2003). Effect of brassinosteroids on certain biochemical parameters in groundnut (*Arachis hypogaea* L.). Indian J. Plant Physiol. 8(3), 313–315.

Rai, V.K. (2002). Role of amino acids in plant responses to stresses. Biol. Plant. 45(4), 481–487. https://doi.org/10.1023/a:1022308229759

Rönsch, H., Adam, G., Matschke, J., Schachler, G. (1993). Influence of (22S,23S)-homobrassinolide on rooting capacity and survival of adult Norway spruce cuttings. Tree Physiol. 12(1), 71–80. https://doi.org/10.1093/treephys/12.1.71

Rosas-Saavedra, C., Stange, C. (2016). Biosynthesis of carotenoids in plants: enzymes and color. Carot. Nature 79, 35–69. https://doi.org/10.1007/978-3-319-39126-7_2

Rosen, H. (1957). A modified ninhydrin colometric analysis for amino acids. Arch. Biochem. Biophys. 67(1), 10–15. https://doi.org/10.1016/0003-9861(57)90241-2

Siddiqui, H., Yusuf, M., Faraz, A., Faizan, M., Sami, F., Hayat, S. (2018). 24-Epibrassinolide supplemented with silicon enhances the photosynthetic efficiency of *Brassica juncea* under salt stress. S. Afr. J. Bot. 118, 120–128. https://doi.org/10.1016/j.sajb.2018.07.009

Siedlecka, M. (2010). Skrypt do ćwiczeń z fizjologii roślin. University of Warsaw. Department of Molecular Plant Physiology, Warsaw, 28–29.

Tanveer, M., Shahzad, B., Sharma, A., Biju, S., Bhardwaj, R. (2018). 24-Epibrassinolide; an active brassinolide and its role in salt stress tolerance in plants: A review. Plant Physiol. Biochem. 130, 69–79. https://doi.org/10.1016/j.plaphy.2018.06.035

Tribulato, A., Toscano, S., Di Lorenzo, V., Romano, D. (2019). Effects of water stress on gas exchange, water relations and leaf structure in two ornamental shrubs in the Mediterranean area. Agronomy 9(7), 381. https://doi.org/10.3390/agronomy9070381

Vardhini, B.V. (2014). Brassinosteroids' role for amino acids, peptides and amines modulation in stressed plants – a review. In: Plant adaptation to environmental change: significance of amino acids and their derivatives. Anjum N. A., Gill S. S., Gill R. (eds). CAB International, 300–316. https://doi.org/10.1079/9781780642734.0300

Wei, Z., Li, J. (2016). Brassinosteroids regulate root growth, development, and symbiosis. Mol. Plant 9(1), 86–100. https://doi.org/10.1016/j.molp.2015.12.003

Xia, X.-J., Huang, L.-F., Zhou, Y.-H., Mao, W.-H., Shi, K., Wu, J.-X., Asami, T., Chen, Z., Yu, J.-Q. (2009). Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus*. Planta 230, 1185–1196. https://doi.org/10.1007/s00425-009-1016-1

Yu, J.Q., Huang, L.F., Hu, W.H., Zhou, Y.H., Mao, W.H., Ye, S.F., Nogués, S., (2004). A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativu*s. J. Exp. Bot. 55(399), 1135–1143. https://doi.org/10.1093/jxb/erh124

Zhu, T., Deng, X., Zhou, X., Zhu, L., Zou, L., Li, P., Zhang, D., Lin, H. (2016). Ethylene and hydrogen peroxide are involved in brassinosteroid-induced salt tolerance in tomato. Sci. Rep. 6(1), 1–15. https://doi.org/10.1038/srep35392