The Effect of Brassinosteroids on Rooting of Stem Cuttings in Two Barberry (Berberis thunbergii L.) Cultivars

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Abstract: Brassinosteroids are a group of over seventy steroid compounds whose discovery in lower and higher plant organisms created new possibilities of plant growth control. The aim of the work was to evaluate the effect of two brassinosteroids: brassinolide (BL) and 24-epibrassinolide (24epiBL) as compared to the auxin rooting enhancer indole-3-butyric acid (IBA), on the rooting of stem cuttings in two Thunberg’s barberry cultivars ‘Maria’ and ‘Red Rocket’. The cuttings were sprayed with water solutions of growth regulators: IBA (200 mg L⁻¹), 0.05% BL or 24epiBL, as well as with a combination of each of brassinosteroids with the auxin while the control cuttings were sprayed with water. In both cultivars brassinosteroids positively affected a degree of rooting and root length. Their application resulted in elevated contents of chlorophyll, total soluble sugars, free amino acids, hydrogen peroxide and catalase activity. Brassinosteroids were more effective when combined with the auxin than when used singly.

Keywords: growth regulators; brassinolide; 24-epibrassinolide; organic compounds; propagation; rooting

1. Introduction

Plant growth regulators are organic compounds modifying physiological functions of plants which enhance or limit plant growth and development [1]. In nursery production auxins are commonly used. They participate in all life processes so they are considered as the most important plant hormones. They inhibit development of dormant buds, are responsible for phototropism, stimulate ethylene production [2], decide stem elongation and conductive tissue development. Auxin as such initiates the development of new root primordia by stimulating mitosis and further cell development [3,4].

The application of diluted or concentrated auxin solutions has become common. This method of application gained popularity as more information about specific concentrations suitable for given plant species became available. Additionally, economical aspects speak for using this method of hormonization as spraying cuttings with water auxin solutions is simpler and quicker than using rooting powders [5,6].

Brassinosteroids are a new group of growth regulators. The first identified brassinosteroid was a brassinolide isolated in 1979 from pollen grains of rape (Brassica napus L.) [7]. Brassinosteroids are now regarded as the sixth group of plant hormones [8]. The group contains over seventy steroid compounds found both in lower and higher plant organisms, in pollen grains, roots or flowers [9].

The following compounds are in brassinosteroid group: brassinolide (BR1 or BL), (BR2), dolicholide (BR3), teasterone (BR8), 28-norbrassinolide (BR14), 28-homobrassinolide (BR17), 24-epibrassinolide (BR27 or 24epiBL), 28-nortyphasterol (BR49), sekasterol (BR53). Three of them—brassinolide, 24-epibrassinolide and 28-homobrassinolide—are used as rooting enhancers [10]. They can be applied by spraying leaves. For the foliar application efficacy, a leaf structure may be crucial. The leaf surface is covered with a cuticle layer,
in which there may be wax layers, which are considered to be a particularly difficult permeable component for growth regulators [7].

The discovery of brassinosteroids created new possibilities for the control of plant growth and development. During the whole growth period, and especially during rooting of leafy cuttings, stress conditions occur such as periodical water deficit or sudden temperature changes. Brassinosteroids can help to overcome such stresses as they are able to increase relative water content, chlorophyll concentration or photosynthesis rate [11,12].

Interactions between different hormones are very important for their action in plants. Brassinosteroids can control plant growth and development, they affect translocation of auxins or change their activity [13]. Some physiological studies have shown that when used jointly with auxins they give better effects than when used singly. A strong synergism between a brassinosteroid and an auxin was found in tests of adzuki bean (*Vigna angularis* Willd.), pea (*Pisum sativum* L.) and bean (*Phaseolus vulgaris* L.) [14].

The barberry (*Berberis thunbergii* L.) cultivars used in the experiment—‘Maria’ and ‘Red Rocket’—are shrubs with decorative foliage, very resistant to air pollution and low temperature so they are important in urban green areas. They are often used as hedges or form compositions in town squares, residential areas and home gardens [15].

The aim of this work was to evaluate the effect of brassinosteroids: Brassinolide (BL) and 24-Epibrassinolide (24epiBL) as well as auxin indole-3-butyric acid (IBA) on the rooting of stem cuttings in two Thunberg’s barberry cultivars ‘Maria’ and ‘Red Rocket’. Changes in the contents of some compounds in treated leaves have also been followed in order to compare the action brassinosteroids with that of the commonly used auxin rooting enhancer IBA.

2. Material and Methods

Semi lignified two-nodal stem cuttings were prepared from shoots harvested from four-year-old stock plants growing in the field. Cuttings were rooted in styrofoam boxes. They were inserted to the depth of 2 cm into a mixture of peat and perlite (2:1 v/v), pH 5.0, covered with a 0.5 cm layer of coarse sand. The experiment consisted of six treatments (Table 1). Each treatment consisted of three replications and each of them contained 20 cuttings. The cuttings were sprayed with water solutions of 200 mg·L\(^{-1}\) IBA (Sigma Aldrich\textsuperscript{®}, St. Louis, MO, USA) or 0.05% Brassinolide or 24-Epibrassinolide (Cayman Chemical Company, Ann Arbor, MI, USA) or combined brassinolides with auxin IBA. The auxin and brassinosteroids were dissolved in 1 mL 96% ethyl alcohol, then they were diluted with water to 1 L. Control plants were sprayed with water containing the same alcohol volume.

| No. of Treatment | Methods of Cuttings Treatment                                      |
|------------------|-------------------------------------------------------------------|
| 1                | Control “0” 1 spraying with distilled water                        |
| 2                | spraying with IBA 200 mg·L\(^{-1}\)                               |
| 3                | spraying with Brassinolide (BL) 0.05%                             |
| 4                | spraying with Brassinolide (BL) 0.05% + IBA 200 mg·L\(^{-1}\)     |
| 5                | spraying with 24-Epibrassinolide (24epiBL) 0.05%                 |
| 6                | spraying with 24-Epibrassinolide (24epiBL) 0.05% + IBA 200 mg·L\(^{-1}\) |

Flats with treated cuttings and the controls were placed in plastic tunnels equipped with automatic watering and mist systems as well as with shading devices. During the first two weeks, the cuttings were protected against the sun with an opaque foil and a shading cloth, which reduced the access of sunlight by 50%. Every week, cuttings were sprayed against *Botrytis* with 0.1% Thiophanate-methyl or Fenhexamid.
2.1. Evaluation of Cuttings

The percentage of rooted cuttings and 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). The percentage of rooted cuttings was also calculated—only the cuttings with a root system within the scale range of 2–5 were regarded as rooted and counted.

Table 2. Evaluation scale of the root development.

| Characteristics 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     |

2.2. Biochemical Analyses

For biochemical analyses, leaves from 20 cuttings per treatment were sampled at the end of the experiment. They were finely chopped and mixed, and 0.5 g samples were used for the measurements. Triplicate extracts were prepared for each treatment and three measurements were done for each extract producing nine readings for each data point. The total chlorophyll content (chlorophyll a + b) was analyzed according to Lichtenthaler and Wellburn [16]. Total soluble sugars were determined by the colorimetric method according to Dubois et al. [17], free amino acids were measured by the method of Rosen [18]. Hydrogen peroxide was measured according to Pick and Keisari [19]. The catalase activity was analyzed according to Goth [20]. Absorbance was measured with the spectrophotometer UV-1601 PC (Shimadzu, Columbia, MD, USA).

2.3. Statistical Analyses

All results were subjected to the analysis of variance using Statgraphics Centurion XVI (Statgraphics Technologies, Inc., The Plains, VA, USA), after the Shapiro–Wilk test. Arcsine transformation was performed for all experimental data taken in percentages before subjecting them to statistical analysis [21]. To compare the means, percentages of rooted cuttings were transformed according to Bliss. Experimental data were subjected to two-way analysis of variance and then to Tukey’s multiple range test to separate the means at the significance level of $p \leq 0.05$ [22].

3. Results

3.1. Rooting of Cuttings in Berberis thunbergii ‘Maria’ and ‘Red Rocket’

Generally, all the growth stimulators positively affected parameters of rhizogenesis (Table 3). As for percentage of rooting, the barberry ‘Maria’ cuttings rooted in over 90% even without any treatment. Use of any brassinosteroid increased it by ~5% relative to the control while IBA gave no effect. However, a joint application of the auxin and a brasssinosteroid increased the percentage of rooted cutting to over 98%. As for a degree of rooting, the best root development was observed after the a joint application of each of the brassinosteroids with IBA or Brassinolide (BL) used alone. All the treatments increased root length, but roots were the longest in cuttings treated with the combinations of either brassinosteroid with IBA where they attained 4.8 cm, i.e., 123% of the control.

In cv. ‘Red Rocket’, the rooting percentage was also high and a joint application of IBA and brassinosteroids resulted in 100% rooting while the results of treatments with three growth regulators used separately did not differ from the control. As for rooting degree, a significant improvement in root ball development was found only after the combined spraying with 24epiBL and IBA. The use of 24epiBL—either alone or with IBA—resulted in the longest roots—114% or 117% of the control, respectively. Root length was also...
significantly increased (by 10%) due to application of the auxin—either separately or jointly with Brassinolide (BL) (Table 4).

Table 3. Effect of brassinosteroids and auxin indole-3-butyric acid (IBA) on the rooting of cuttings in Berberis thunbergii 'Maria'.

| Parameter            | Control | IBA  | BL   | BL + IBA | 24epiBL | 24epiBL + IBA |
|----------------------|---------|------|------|----------|---------|---------------|
| Rooted cuttings (%)  | 91.7 ± 2.8 a * | 91.7 ± 2.7 a | 96.7 ± 2.8 b | 98.3 ± 2.7 b | 98.3 ± 2.8 b | 98.3 ± 2.8 b |
| Rooting degree       | 3.9 ± 0.3 a    | 4.1 ± 0.2 ab  | 4.4 ± 0.3 bc | 4.6 ± 0.1 c | 4.2 ± 0.3 ab  | 4.4 ± 0.3 bc  |
| Root length (cm)     | 3.9 ± 0.1 a    | 4.1 ± 0.1 b   | 4.3 ± 0.3 b  | 4.8 ± 0.2 c | 4.2 ± 0.3 b   | 4.8 ± 0.3 c   |

* Means in the line followed by the same letter do not differ significantly at α = 0.05. Means ± standard deviation within a row followed by the same letter are not significantly different according to Tukey’s multiple range test at α = 0.05.

Table 4. Effect of brassinosteroids and auxin IBA on rooting of cuttings in Berberis thunbergii 'Red Rocket'.

| Parameter            | Control | IBA  | BL   | BL + IBA | 24epiBL | 24epiBL + IBA |
|----------------------|---------|------|------|----------|---------|---------------|
| Rooted cuttings (%)  | 93.3 ± 25.7 a | 96.7 ± 5.7 ab | 98.3 ± 2.8 ab | 100.0 ± 0.0 b | 98.3 ± 2.9 ab | 100.0 ± 0.0 b |
| Rooting degree       | 4.1 ± 0.1 a * | 4.2 ± 0.2 ab  | 4.1 ± 0.2 a  | 4.4 ± 0.3 ab  | 4.3 ± 0.4 ab  | 4.6 ± 0.1 b   |
| Root length (cm)     | 4.2 ± 0.1 a    | 4.6 ± 0.2 b   | 4.3 ± 0.1 a  | 4.6 ± 0.2 b   | 4.8 ± 0.1 c   | 4.9 ± 0.1 c   |

* Means in the line followed by the same letter do not differ significantly at α = 0.05. Means ± standard deviation within a row followed by the same letter are not significantly different according to Tukey’s multiple range test at α = 0.05.

3.2. Biochemical Analyses

In the cuttings of both cultivars, the treatments significantly affected the contents of some organic compounds and catalase activity. The joint application of the auxin and 24-epibrassinolide (24epiBL) resulted in the increase in chlorophyll content in cuttings of cv. ‘Maria’ while the use of IBA or Brassinolide (BL) alone was ineffective. In ‘Red Rocket’ the chlorophyll level was almost 10% higher relative to the control due to spraying cuttings with 24-epibrassinolide (24epiBL)—singly or together with IBA. The treatment with Brassinolide (BR)—alone or with the auxin—gave increase in chlorophyll by 14% and 19%, respectively (Table 5).

Table 5. Effect of brassinosteroids and auxin IBA on chlorophyll, total soluble sugar, free amino acids, hydrogen peroxide (H₂O₂) contents, and catalase activity in cuttings Berberis thunbergii ‘Maria’ and ‘Red Rocket’.

| Parameter            | Cultivar              | Control | IBA  | BL   | BL + IBA | 24epiBL | 24epiBL + IBA |
|----------------------|-----------------------|---------|------|------|----------|---------|---------------|
| chlorophyll [mg g⁻¹ d.w.] | B. th. Maria          | 3.1 ± 0.1 a * | 3.2 ± 0.1 a | 3.2 ± 0.2 ab | 3.3 ± 0.3 ab | 3.4 ± 0.1 b | 3.5 ± 0.1 a b |
|                      | B. th. Red Rocket     | 3.6 ± 0.1 a  | 3.7 ± 0.1 a | 4.1 ± 0.1 cd | 4.3 ± 0.0 d  | 3.9 ± 0.1 b | 4.0 ± 0.2 bc  |
| total soluble sugar [mg g⁻¹ d.w.] | B. th. Maria          | 76.2 ± 2.5 a  | 84.9 ± 8.8 ab | 99.6 ± 11.1 c | 130.6 ± 12.0 d | 96.5 ± 2.3 bc | 96.4 ± 2.4 bc  |
|                      | B. th. Red Rocket     | 66.9 ± 2.2 a  | 82.9 ± 1.3 ab | 138.1 ± 8.5 d | 89.1 ± 5.1 b  | 90.3 ± 21.9 b | 101.7 ± 1.9 c |
| free amino acids [µmol leucine g⁻¹ d.w.] | B. th. Maria          | 525.0 ± 21.6 a | 559.3 ± 32.3 ab | 764.1 ± 20.7 c | 790.3 ± 30.9 c | 593.2 ± 15.7 b | 798.1 ± 20.5 c  |
|                      | B. th. Red Rocket     | 454.4 ± 15.6 a | 608.9 ± 18.7 b | 640.0 ± 21.9 b | 746.2 ± 8.7 d  | 619.5 ± 7.1 b | 701.1 ± 24.7 c |
| H₂O₂ [µg g⁻¹ d.w.]   | B. th. Maria          | 172.2 ± 3.5 a  | 160.2 ± 2.3 a | 244.5 ± 3.2 b | 255.9 ± 7.8 b  | 248.6 ± 3.8 b | 242.2 ± 18.1 b |
|                      | B. th. Red Rocket     | 297.2 ± 6.9 a  | 402.5 ± 9.2 c | 311.2 ± 4.4 ab | 323.1 ± 1.8 b  | 348.8 ± 9.3 c | 397.3 ± 2.3 d  |
| catalase [mkat g⁻¹ d.w.] | B. th. Maria          | 1757.2 ± 439.8 a | 2790.5 ± 369.5 b | 2599.5 ± 336.6 b | 3577.8 ± 371.3 c | 2420.1 ± 320.6 b | 3487.6 ± 383.1 c  |
|                      | B. th. Red Rocket     | 3492.1 ± 129.1 a | 6937.3 ± 565.8 b | 11986 ± 570.1 c | 14111.5 ± 462.1 d | 12130.9 ± 462.1 c | 14467.3 ± 200.2 d |

* Means in the line followed by the same letter do not differ significantly at α = 0.05. Means ± standard deviation within a row followed by the same letter are not significantly different according to Tukey’s multiple range test at α = 0.05.

Due to treatment with 24-epibrassinolide (24epiBL)—alone or jointly with IBA—the total soluble sugar content increased in ‘Maria’ cuttings by 27% relative to the control (Table 5). The use of Brassinolide (BL) (alone or together with IBA) also elevated sugar levels as compared with the control, by 31 and 74%, respectively. No significant effect of IBA on sugar content was found, similarly as in ‘Red Rocket’. However, in the latter cultivar the sugar concentrations in cuttings increased significantly after the joint treatment with the auxin and BL or 24epiBL—by 33% and 52%, respectively. Spraying cuttings with
the BL solution almost doubled the sugar content, which was significantly higher than after 24epiBL application.

Generally, the content of free amino acids in ‘Maria’ cuttings was increased by the treatments as compared to the control. This increase was 13% after spraying cuttings with 24-epibrassinolide (24epiBL) while it ranged between 46% and 52% after the joint treatment with the auxin plus any of brassinolides as well as Brassinolide (BL) alone. Additionally, in ‘Red Rocket’, free amino acids increased after spraying with the preparations under study. IBA application resulted in a 34% increase while both brassinosteroids gave rise by 36–40% relative to the control. Higher increases in concentrations of free amino acids were found in cuttings treated with the mixtures of IBA with the brassinosteroids—54% and 64% for BL and 24epiBL, respectively (Table 5).

In cuttings of cultivar ‘Maria’ treated with brassinosteroids (alone or in combination with IBA) the level of hydrogen peroxide increased relative to the control by 40–49%. The same tendency was observed in ‘Red Rocket’, especially in cuttings treated with IBA and 24-epibrassinolide where H₂O₂ increased by nearly 34%. About 17% increase was found in cuttings sprayed with 24-epibrassinolide combined with the auxin (Table 5). Catalase activity increased in barberry cuttings after treatments with all the preparations (Table 5). In ‘Maria’, IBA application resulted in almost a 59% increase while the use of 24epiBL and BL gave increase in catalase activity by 48 and 38%, respectively. In the second cultivar the treatment with IBA induced a two-fold increase in enzyme activity while in cuttings sprayed with 24epiBL or BL this activity increased 3.5 times. The application of any of the brassinosteroids together with IBA resulted in the four-fold increase in catalase activity (Table 5).

4. Discussion

Rooting enhancers are very important in plants’ vegetative propagation. The most popular are auxins commonly used to stimulate the rooting of cuttings. They are applied either as rooting powders or water solutions [23]. Pacholczak et al [24] confirmed a high efficacy of IBA water solutions in the rooting of stem cuttings in three ninebark (Physocarpus opulifolius (L.) Maxim.) cultivars. This was also observed here in two barberry cultivars (Berberis thunbergii ‘Maria’ and ‘Red Rocket’) where root length increased due to spraying the cuttings with water IBA solution. Additionally, Hou et al. [25] reported an increase in root number and length in azalea cuttings. In Rhododendron austrinum (Small) Rehder. root length in cuttings was doubled due to IBA application [26].

The application of two compounds from brassinosteroid group—Brassinolide and 24-epibrassinolide—positively affected rooting in stem cuttings of both barberry cultivars under study. Earlier, their efficacy in rhizogenesis was reported by Swamy and Rao [8] who found an increased root number and their better development in the cuttings of geranium and coleus. Additionally, in cuttings of indian coleus (Plectranthus barbatus Benth. ex G.Don) the use of 28-homobrassinolide and 24-epibrassinolide improved root induction and development [27], and treated cuttings produced twice as many roots as the untreated control.

Results of investigations on brassinosteroids indicate that the compounds control the elongation processes in plants [28,29]. According to Kim et al. [30] brassinosteroids increased root length in cuttings of basil (Ocimum basilicum L.), tomato (Solanum lycopersicum L.) and chrysanthemum (Chrysanthemum indicum L.). In this experiment the longest roots (ca 1 cm longer than in control) were produced by cuttings of barberry cv. ‘Maria’ treated with the solutions BL+IBA or 24epiBL+IBA, while 24epiBL and 24epiBL+ IBA were effective in cv. ‘Red Rocket’. The synergisms of brassinosteroids with the auxin was evident: IBA when used alone did not affect root elongation while 24epiBL as such promoted it (in cv. ‘Red Rocket’). Joint application of both growth regulators gave in the best effects for all the rooting parameters. Synergism in the action of brassinosteroids and auxin has already been reported [31,32]. Earlier, Bao et al. [33] found that using a brassinosteroid with an auxin positively affected root development in thale cress (Arabidopsis thaliana (L.).
In this work, the contents of several organic compounds and parameters of oxidative stress in cuttings have been determined. The application of brassinosteroids not only affected the rooting of barberry cuttings but also resulted in changes in their biochemical composition relative to untreated control.

Brassinosteroids affect defense mechanisms in plants by increasing their tolerance to stresses (in cuttings those due to detachment from the mother plant). They increase chlorophyll content which improves the photosynthesis rate [34]. Such an effect was seen in barberry after application of both compounds under study, especially in cv. ‘Red Rocket’ where chlorophyll content increased by 0.5 mg g⁻¹ DW as compared to the control. Such positive effects of 24-epibrassinolide on chlorophyll content were earlier reported by Yu et al. [35] in cucumber seedlings and by Dalio et al. [36] in pigeon pea (Cajanus cajan L.) seedlings.

Apart from chlorophyll concentration, contents of carbohydrate—as products of photosynthesis—should be considered when assessing photosynthetic activity in plant. It seems that as hydrogen peroxide damages cells when present in high concentration it should also contribute to chlorophyll degradation and decrease photosynthesis parameters. However, the opposite effects have been observed: according to Kato and Shimizu [37] H₂O₂ may protect chloroplast ultrastructure to preserve photosynthetic pigments and stimulate photosynthesis. Additionally, the recent results obtained on mistletoe fig (Ficus deltoidea Jack) confirmed that the application of H₂O₂ increased the leaf chlorophyll content, net photosynthetic rate and stomatal conductance [38]. During rooting, the cutting needs much energy to regenerate new tissues and organs, therefore it is crucial for a rooting enhancer not to use it but to support its production [39]. Brassinosteroids tested here seem to fulfill this postulate as during barberry rooting the amount of sugars in cuttings treated with either compound was higher relative to the control. The highest amount was determined in cuttings of cv. ‘Red Rocket’ sprayed with BR where it attained 138 mg g⁻¹ DW, i.e., the content over twice as high as in untreated cuttings. In Arachis hypogaea L. the carbohydrate level also increased after brassinosteroid application [40]. According to Xia et al. [41] the application of these growth regulators in cucumber resulted in the increased sugar concentration in leaves which improved a general plant condition, which was also true for barberry.

Free amino acids are important for forming new tissues as they are responsible for the regeneration of tissues subjected to stresses [42,43]. In barberry cuttings treated with BL or 24epiBL those of cv. ‘Maria’ contained more free amino acids than of cv. ‘Red Rocket’. In both cultivars a joint application of brassinosteroids with the auxin resulted in the highest content of free amino acids. Similar observation was made by Li et al. [44] in tea (Camellia sinensis (L.) Kuntze) leaves sprayed with 24-epibrassinolide, Pustovoitova et al. [45] in cucumber (Cucumis L.) and Chhetri and Roy [46] in cuttings of eight rhododendron species.

Apart from appropriate pools of building materials, a well-balanced antioxidant system is important for rhizogenesis. High concentration of hydrogen peroxide—a reactive oxygen species (ROS)—negatively affects different processes occurring in plants. Its amount increases under stress conditions such as water deficit or high salt concentration [13]. This happened in both barberry cultivars regardless of the use of a rooting stimulator. The only small decrease in H₂O₂ was observed in ‘Maria’ cuttings treated with 200 mg l⁻¹ IBA. Results of other investigators confirm a relationship between application of brassinosteroids and rise in H₂O₂, for example Zhu et al. [47] found that the content of hydrogen peroxide in tomato plants treated with a hormone of this group almost doubled. However, 24-epibrassinolide can lower H₂O₂ concentration in plants, like in pepper (Capsicum annuum L.) subjected to water stress [48]. Additionally, its application on young flax seedlings decreased H₂O₂ level [49]. According to Begnamnia et al. [50] the use of brassinolide in several concentrations on tomato plants (Lycopersicon esculentum Mill.) subjected to drought resulted in a considerable fall in hydrogen peroxide in leaves. It is, however, difficult to establish a correct relationship between stress and amounts of H₂O₂ as it is not clear if the plant antioxidative system has been turned on. Hydrogen...
peroxide is not only a signal molecule in stress reactions but it also has a proven positive effect on rooting. In barberry cuttings its highest content was found in the treatment with IBA and 24epiBL+IBA which assured the best rooting. Numerous reports indicate that H₂O₂ may be used as a specific marker in an early phase of rhizogenesis. Accumulation of free H₂O₂ may be a follow-up event resulting from the adventitious root formation in cuttings [51]. The hydrogen peroxide solution was successfully used to stimulate rooting of two olive (Olea europaea L.) cultivars [52]. Plant response to an increased H₂O₂ content is a rise in catalase activity in plant tissues. Brassinosteroids as hormones which help plant to survive under stress conditions stimulate activity of antioxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT) [53–55]. Such dependence was found in both barberry cultivars, especially in cuttings treated with brassinosteroids: in cv. ‘Maria’ and ‘Red Rocket’ the increase in catalase activity was two- and four-fold, respectively. According to Cai et al. [56] who used 24-epibrassinolide on plants of Rhododendron delavayi Franch., a range of plant response to a treatment depended on a hormone concentration used at the beginning of the experiment. The experiments done in tissue cultures on peanut (Arachis hypogaea L.) genotypes confirmed that the use of brassinolide definitely stimulated activity of the stress enzyme which is catalase [55]. Similar results were obtained by Lima and Lobato [57] who observed a three-fold increase in catalase activity in germinated seeds of cowpea (Vigna unguiculata L.) treated with 100 nM 24-epibrassinolide.

5. Conclusions

Brassinolide and 24-epibrassinolide not only improved the rooting of barberry cuttings (Berberis thunbergii ‘Maria’ and ‘Red Rocket’) but also affected their contents of some organic compounds. In both cultivars, brassinosteroids positively affected a degree of rooting and root length.

Their application resulted in elevated contents of chlorophyll, total soluble sugars, free amino acids, hydrogen peroxide and catalase activity. Brassinosteroids were more effective when combined with the auxin than when used singly.

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