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RESEARCH ARTICLE

Enhancing rooting efficiency and nutrient uptake in *Rosa damascena* Mill. cuttings: insights into auxin and cutting type optimization

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Abstract

Auxin application plays a crucial role in successfully propagating and cultivating *Rosa damascena* Mill., a valuable plant species with cultural and economic significance. In this study, we aimed to investigate the effects of auxin dose and leaf presence on rooting success and nutrient uptake efficiency in *Rosa damascena* cuttings. Our results demonstrated that applying IBA significantly improved rooting success and nutrient absorption capacity, with a concentration of 1000 ppm being the most effective, resulting in a remarkable rooting % (48 ± 6%), increased root length (2.52 ± 0.25 cm) and improving leaf area (70.18 ± 5.10 cm^2). The inclusion of leaves on cuttings has greatly magnified root success (90 ± 10%), leaf area (98.86 ± 17.86 cm^2) and nutrient absorption efficiency, highlighting the vital role of leaves in early root development. Our findings provide valuable insights into the development of sustainable and productive cultivation methods for this significant plant species. Furthermore, our investigation emphasized the importance of optimizing auxin application, with leafy cuttings treated with 1000 ppm of auxin showing the most promising results regarding growth attributes, nutrient assimilation and survival rate.

Keywords

Indole-3-butyric acid (IBA), leaf presence, nutrient uptake, rooting success, *Rosa damascena,* sustainable cultivation

Introduction

Rosa damascena Mill., a hybrid rose belonging to the *Rosaceae* family, holds significant historical importance (1). Cultivated widely in the Mediterranean basin, Morocco is a leading producer of Damask rose (1). The flowers of *R. damascena* have been used for medicinal and culinary purposes for over a thousand years, exhibiting therapeutic properties, including anti-inflammatory, anti-depressant effects, improved blood circulation and endocrine regulation (2). In addition, the plant's flagship products, rose water, rose oil and dry flower buds are widely used in the luxury perfumery and aroma industries (3).

Traditional methods of propagating Damask rose, such as suckering, can lead to reduced yield, quality and delayed flowering (4). Therefore, asexual propagation methods, such as cutting, have become increasingly popular (5). However, the success of this method is highly dependent on intrinsic factors, such as genetic potential, nutritional reserves and hormonal balance as well as extrinsic factors, including temperature, humidity, light and plant health (6). Failure to manage these factors can lead to poor rooting success and negatively impact the propagation and cultivation of Damask rose (7).

Plant hormones play a significant role in plant growth and development; among them, auxins have been shown to enhance rooting in stem cuttings (5). The most effective auxin for promoting root initiation and adventitious root production in stem cuttings is indole-3-butyric acid (IBA) (8). Adventitious roots, which are formed from non-root tissues, emerge from the callus and are considered the primary roots of the cuttings (9). These roots contain a high concentration of auxins, which play a critical role in their development and establishment (10). In addition, auxins also influence the architecture and morphology of roots, which can significantly impact the seedling's absorption capacity and nutritional status (11). Studies have shown that auxins regulate the development of lateral roots, which can increase the root surface area and enhance nutrient absorption (12). Auxins also influence the formation of root hairs, can further increase the area of the roots for nutrient uptake (13). Overall, the role of auxins in promoting rooting and influencing root architecture and morphology highlights their importance in the propagation and cultivation of plants. Understanding the effect of auxins and their optimal dosage is essential for developing effective propagation methods and improving crop yield and quality.

The presence or absence of leaves can significantly impact the rooting of *R. damascena* cuttings (14). While leaves are essential for photosynthesis and nutrient production, they can also lead to excessive transpiration and reduce the availability of resources for root development (15). Thus, removing some or all of the leaves from the cutting can help to promote rooting by reducing water loss and allowing more resources to be allocated towards root growth (16). However, removing too many leaves can also have adverse effects on the cutting's ability to root and establish itself. Therefore, finding the right balance between leaf presence and absence is crucial for the successful propagation of *R. damascena.*

The present study endeavors to elucidate the optimal dose of auxin for the successful rooting of *R. damascena* cuttings and assess the nutrient uptake efficiency of the ensuing roots. The application of auxins, mainly IBA, to promote root induction and adventitious root formation in stem cuttings has been widely recognized. Therefore, this investigation aims to determine the most effective auxin concentration to ensure an accurate and effective rooting process. Furthermore, this study will also explore the effect of cutting type on rooting efficacy and nutrient absorption potential. By augmenting our understanding of the interplay between auxins and cutting type in root growth and nutrient assimilation, this research will provide valuable insights into the propagation and cultivation of Damask rose, a plant species of substantial historical and economic significance.

Materials and Methods

Experimental details

To investigate the effects of different doses of auxins and cutting types (leafy and non-leafy) on rooting, bud break, and nutrient absorption capacity, a trial was conducted on uniform cuttings of *R. damascena*. Cuttings of 17-20 cm in length and 7-10 mm in diameter with at least 4 nodes were selected from the middle part of vigorously grown shoots of mother plants of *R. damascena*. The experiment was conducted in a miniature greenhouse under controlled conditions at the Institute of Agronomy and Veterinary Hassan II, Agadir, Morocco (30 °35' 31 ''N, -9 °47' 71'' E). The study was designed using a complete randomized design (CRD) with 5 replications for every treatment. Each treatment consisted of 10 cuttings to ensure the reliability of the results.

Cutting

To avoid disrupting the plant's physiological state during the cuttings collection and to prevent stress and dehydration of the cuttings themselves, both leafy and non -leafy cuttings were collected early in the morning. Before planting, a 1 cm fragment of the basal part of each cutting was removed and the cuttings underwent a 5-second immersion in a 3% sodium hypochlorite solution, followed by a rinse with pure distilled water. Next, the basal 3 cm of the cuttings were treated with Indole-3-butyric acid (IBA) powder at 3 different concentrations: 0 ppm, 1000 ppm and 10000 ppm. The cuttings were then rapidly soaked in the IBA solution for 20 seconds before immediately being planted into a 77-cell plug tray filled with a substrate mixture of perlite, peat and sterilized sand at a ratio of 3:1:1 (v:v:v). This substrate mixture provides good aeration and water retention during the rooting of plant cuttings. Table 1 summarizes the physico-chemical characteristics of the substrate used for the cuttings.

Table 1. Physico-chemical properties of substrate in the experiment

Property	Value
Soil pH	7.08
Available N	57.1
Available P2O5	27.8
Available K2O	195.8
Organic matter	1.4
Ec	1.7
Soil texture	Sandy silty
Sand	68.8
Silt	22.3
Clay	8.9

Note: ±: Standard deviation of three replicates; N, P2O5, K2O(ppm): parts per million; Organic matter, Sand, Silt, Clay (%): Percentage; Ec (mS/cm).

The plants propagated from the cuttings were transferred to a small-scale greenhouse with controlled environmental conditions. The temperature inside the greenhouse was kept within a range of 21 °C to 25 °C, while the temperature of the bed fluctuated between 24 °C and 28 °C. The relative humidity was kept at 100% for the duration of the experiment. A data logger (TZone-TempU03) was used to monitor the temperature and humidity levels. To optimize growth conditions, the substrate was consistently irrigated at a weekly interval, ensuring regular and sufficient moisture supply.

Growth attributes

After 70 days, the effects of the studied factors, namely cutting types and auxin treatments, on root induction and initiation in *R. damascena* were evaluated by examining several parameters. These parameters included the rooting rate, mortality rate, number and type of roots per cutting, number of shoots formed, number of leaflets per leaf and number of leaves per bud. By analyzing these factors, the efficacy of the experimental treatments in promoting the growth and development of the plants could be assessed.

Rooting rate, number of roots per cutting and mortality rate

The rooting rate was determined by comparing the number of cuttings that had developed roots with the number of cuttings that had not at the end of the rooting period. To do this, the seedlings were removed from the substrate, and the root numbers were counted for each cutting that had developed lateral or primary roots. Rooted and unrooted cuttings were assigned a value of 1 and 0 respectively. The rate of rooting was calculated by determining the % of rooted cuttings. To assess the overall health of the cuttings, both the number of rotted or dried cuttings and the number of living cuttings were counted. The mortality rate was determined by calculating the percentage of cuttings that had died.

Number of shoots formed and of leaflets per leaf and number of leaves per bud

After the experiment, the number of shoots that emerged was documented for all cuttings to evaluate the influence of various treatments on bud break and growth. Additionally, the leaf bud count and the number of leaflets per leaf were recorded for each cutting.

Potting

The cuttings that had successfully rooted were carefully extracted from the substrate and transplanted into polyethylene pots with a volume of 0.5 L. The pots were filled with a mixture of perlite, peat and sand in the ratio of (3:2:1 /v; v; v), providing optimal aeration and water retention for plant growth. Table 2 below summarizes the physico-chemical properties of the substrate mixture used during potting.

The seedlings were carefully transferred to a Richel-type greenhouse with a built-in cooling system and placed on a tide table. The temperature inside the greenhouse was maintained between 20 °C and 27 °C, while the humidity ranged from 50% to 80%.

Table 2. Physico-chemical properties of substrate in the experiment

Property	Value
Soil pH	6.5
Available N	98.7
Available P2O5	46.7
Available K2O	247.0
Organic matter	2.1
Ec	1.8
Soil texture	Sandy silty
Sand	61.3
Silt	28.4
Clay	10.1

Note: ±: Standard deviation of three replicates; N, P2O5, K2O (Ppm): parts per million; Organic matter, Sand, Silt, Clay (%): Percentage; Ec (mS/cm)

Root volume and absorption capacity

After a 10-day acclimation period, a random selection of 5 seedlings from each treatment was assessed for the quality of their root system by studying their absorptive abilities under controlled laboratory conditions. Test tubes were sterilized with Ethanol 90%, labeled and filled with a nutrient solution consisting of a soluble fertilizer of (20;20;20, Nitrogen, Phosphorus and Potassium) plus trace elements. Air pumps were used to oxygenate the solution to prevent plant asphyxiation. Before placing the plants in the tubes, all substrate residues were rinsed with tap water to avoid any potential biases in the results. The initial weight (tube + solution) and weight of the tubes with plants and solution were measured, allowing for the determination of the weight variation during the 3-day experiment period. The 3 main parameters evaluated were the absorption and transpiration rate, root volume and leaf surface.

Rate of absorption of nutrient solution

To investigate the absorption rate of nutrients in cuttings with and without leaves, as well as the effect of auxin on nutrient uptake, the absorption rate of roots was measured. This was determined by monitoring the continuous decrease in the weight of nutrient solution in test tubes over 3 consecutive days, with increasing time intervals.

Root volume

To assess the size of the root system, each seedling was carefully placed in a graduated test tube that was partially filled with water. The water displacement method was then used to determine the volume of the root system. This technique is widely used in plant research and permits precise root growth and development measurement.

Leaf surface

Leaf surface area was quantified using the "CANOPEO" software, which accurately measures leaf area % with a predefined scale. First, the conversion from pixels to cm^2 was calculated. A photograph of downward-facing leaves placed on a black background was taken and the image was analyzed to determine the extent of the leaf surface area, quantified as the leaf area index (LAI).

Fresh and dried weight of roots and aerial part measurement

At the experiment's conclusion, the fresh and dry weights of the seedlings' aerial parts were determined by an electronic balance (Nahita blue ®, Auxilab). The roots and aerial parts were weighed separately. The samples were then subjected to oven drying at 50 °C for 48 hrs and their dry weight was measured afterward.

Foliar analysis

After the leaves were subjected to calcination at a temperature of 600 °C, mineral elements were extracted using hydrogen chloride and subjected to analysis. The

Table 3. Propagation results induced by diffrents treatments applied. Means followed by the same letter are not statistically different at p < 5% according to the Tukey test

Treatment IBA×Leafy/non-leafy		Rooting rate in (%)	Survival rate in (%)	Root lenth in (cm)	Roots number	Number of neoformed shoots	Number of leaves per bud	Number of leaflets per leaf
Without leaves	0 ppm	23% ± 10% (c)	30% ± 7% (b)	0,71 ± 0,18 (d)	1,43 ± 0,43 (d)	1,50 ± 0,37 (e)	2,45 ± 0,76 (b)	6,37 ± 0,87 (a)
	1000 ppm	48% ± 6% (b)	40% ± 6% (b)	2,52 ± 0,25 (c)	4,92 ± 0,75 (c)	2,52 ± 0,34 (c)	2,97 ± 0,30 (b)	6,56 ± 0,35 (a)
	10000 ppm	58% ± 17% (b)	40% ± 16% (b)	6,75 ± 0,41 (a)	11,90 ± 1,26 (a)	4,03 ± 0,32 (a)	4,55 ± 0,30 (a)	5,40 ± 0,14 (b)
With leaves	0 ppm	13% ± 9% (c)	23% ± 16% (b)	0,76 ± 0,50 (d)	1,00 ± 0,71 (d)	2,38 ± 0,14 (cd)	2,70 ± 0,19 (b)	6,48 ± 0,18 (a)
	1000 ppm	90% ± 10% (a)	78% ± 10% (a)	4,63 ± 0,19 (b)	7,15 ± 0,55 (b)	3,17 ± 0,21 (b)	4,20 ± 0,19 (a)	6,97 ± 0,06 (a)
	10000 ppm	65% ± 10% (b)	38% ± 9% (b)	2,81 ± 0,31 (c)	5,09 ± 0,55 (c)	1,81 ± 0,33 (de)	1,67 ± 0,07 (c)	6,49 ± 0,26 (a)

total nitrogen content was determined by employing the Kjeldahl method. In contrast, the total phosphorus content was measured by complexing it with molybdate and ammonium vanadate and subsequently using colorimetry. The organic carbon content was determined using the loss on ignition method at 600 °C. Additionally, the K+, Na+, Mg++ and Ca++ ions concentrations were measured using spectrophotometry.

Substrate analysis

The physicochemical properties of the various substrates were analyzed in the laboratory of soil sciences at the National Research Institute of Agriculture (INRA) in Rabat, Morocco. To ensure accuracy, each substrate mixture was analyzed in triplicate. The organic matter content was determined using the Walkley and Black method. Available nitrogen (N) was measured using the Kjeldahl method, available phosphorus (P2O5) was assessed using the Olsen method and available potassium (K2O) was determined using the Mehlich method. The cation exchange capacity was calculated based on the exchangeable bases. Soil texture was determined using a hydrometer, while pH was measured using a glass electrode-pH meter. Electrical conductivity was measured using the saturated paste method, which involves measuring the electrical conductivity of the soil's saturated paste after it has been equilibrated for 24 hrs.

Statistical analysis

All the data collected were analyzed using standard statistical methods, including analysis of variance (ANOVA). The ANOVA was performed in the Minitab statistics software to determine the relationship between the growth attributes of *R. damascena*, rooting, and mortality rates with different treatments studied. This was done to validate the correlation between different doses of auxin and the success of both leafy and non-leafy cuttings. The confidence intervals were set at 95% and p-values were used to determine statistical significance. A p-value of ≤0.05 was considered a significant difference, while a p-value of ≤0.01 was considered a highly significant difference between 2 different treatments. The statistical analysis was conducted with the purpose of obtaining a comprehensive understanding of the effectiveness of diverse treatments in facilitating the successful establishment of root systems in *R. damascena* cuttings.

Results and Discussion

Growth attributes

The outcomes derived from the diverse techniques of vegetative propagation indicate that both the type of cuttings (leafy and non-leafy) and the various treatments of auxin derivatives have a substantial impact on the rooting rate, the survival rate of cuttings, the number and length of roots, the number of new shoots, the number of leaves per bud and the number of leaflets per leaf. These findings demonstrate the importance of selecting the appropriate cutting type and optimal treatment of auxin derivatives in order to fully propagate *R. damascena*. (Table 3)

Rooting rate

The results of this study demonstrate the importance of considering the presence of leaves on cuttings and the dosage of auxin in the rooting process. The statistical analysis revealed a highly significant interaction between these factors in terms of the success rate of the rooting process. Interestingly, the success rate of leafless cuttings increased with the increase of the auxin dose, indicating that adding auxin can compensate for the absence of leaves in promoting rooting. Specifically, the success rate increased from approximately 20% with the 0 ppm dose to 58% with the 10000 ppm dose. However, this trend was not observed in leafy cuttings. Furthermore, it was found that the presence of leaves on cuttings negatively affects the success rate of the rooting process, with leafless cuttings showing a higher success rate than leafy cuttings. The higher energy and resource demands of leaves on the cutting may potentially hinder rooting; nonetheless, when exposed to a 1000 ppm dose, leaves exhibited a higher rooting rate, indicating that beyond a certain threshold, leaves may stimulate rooting through the production of endogenous auxins or other growth-promoting compounds (Fig. 1).

This study's findings align with previous research that has investigated the effect of auxin on rooting success in cuttings (5). Auxins promote cell division and elongation (17), which are critical processes in root formation. The present study further highlights the importance of considering the dosage of auxin in promoting rooting success. One possible explanation for the negative effect of leaves on rooting success is the competition for resources between the leaves and the developing roots (18). The high energy and nutrient demands of leaves (19)

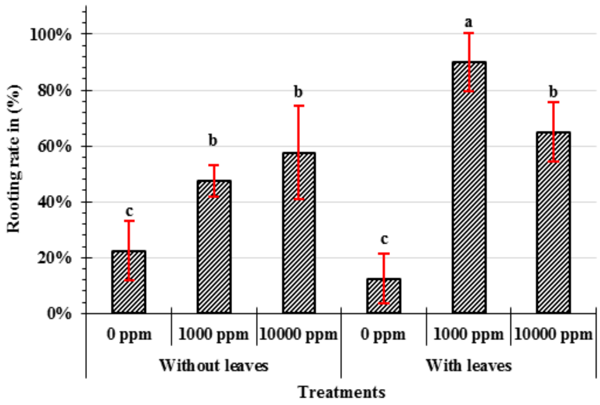

Fig. 1. Rooting rate of cuttings induced by different treatments in (%). Means followed by the same letter are not statistically different at p < 5% according to the Tukey test.

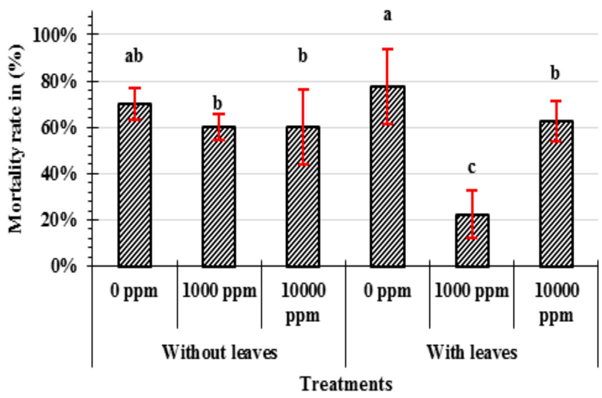

Fig. 2. Mortality rate of cuttings induced by different treatments in (%). Means followed by the same letter are not statistically different at p < 5% according to the Tukey test.

can restrict resource availability for root development, potentially impeding the rooting process. Moreover, a study by (20) found that the presence of leaves on cuttings reduced the expression of genes involved in root formation, suggesting that leaves can inhibit the signaling pathways involved in rooting.

Nevertheless, the observed improvement in rooting rate with the presence of leaves at 1000 ppm auxin dose is an interesting finding. Previous studies have shown that leaves can produce endogenous auxins, which can promote rooting (21). It is possible that the 1000 ppm dose of auxin triggered the production of endogenous auxins in the leaves, leading to the observed improvement in rooting success. In conclusion, the current research highlights the importance of considering the presence of leaves and the dosage of auxin in promoting rooting success in cuttings. While leaves can harm rooting success due to competition for resources, the production of endogenous auxins by leaves can also promote rooting under certain conditions. Further research is needed to explore these processes' underlying mechanisms and optimize rooting protocols for *R. damascena.*

Mortality rate

The results of this study highlight the importance of considering both the presence of leaves and the dosage of auxin in the survival rate of *R. damascena* cuttings. The statistical analysis revealed a highly significant interaction between these factors regarding mortality rate. Interestingly, the leafy cuttings group that received 1000 ppm auxin exhibited the lowest mortality rate of just 20%. In contrast, all other treatments showed a mortality rate above 60%. These findings are depicted in Fig. 2. This suggests that leaves may have a protective effect on the cuttings at a certain threshold of auxin dosage. However, in all other treatments, the mortality rate was found to be significantly high. The presence of leaves on cuttings harmed their survival rate, because leaves require more energy and resources from the cutting, diverting resources away from survival and rooting. Overall, these results suggest that careful consideration of both the presence of leaves and the dosage of auxin is critical in promoting the survival and rooting of *R. damascena* cuttings. Further research is needed to determine the optimal threshold for auxin dosage and the specific mechanisms by which leaves and auxin interact to affect the survival and rooting of plant cuttings.

The results of this study provide important insights into the role of leaves and auxin in the survival rate of *R. damascena* cuttings. The finding that leafy cuttings that received 1000 ppm auxin exhibited the lowest mortality rate is particularly interesting, as it suggests that the presence of leaves may have a protective effect on the cuttings at a certain threshold of auxin dosage. This protective effect of leaves has also been observed in other plant species. For example, a study on *Populus deltoides* cuttings found that leaves improved survival and growth rate, possibly due to the production of growth-promoting compounds by the leaves (22). Another study on *Chrysanthemum morifolium* cuttings found that leaves reduced water loss and improved survival rate, possibly due to the production of cytokinins by the leaves (23).

However, in all other treatments, the mortality rate was significantly high. This is supported by a study on *Populus tremula* cuttings, which found that removing leaves increased rooting and improved survival rates (24). In terms of auxin dosage, the findings of this study suggest that a careful balance must be struck to promote survival and rooting. Previous research has found that high doses of auxin can negatively affect rooting, possibly due to the inhibition of endogenous auxin production or the accumulation of toxic levels of auxin (25). Therefore, it is important to determine the optimal threshold for auxin dosage to promote survival and rooting. This may depend on a variety of factors, including the specific plant species, the type of cutting and the environmental conditions.

In conclusion, the results of this study highlight the complex interplay between leaves and auxin in the survival and rooting of plant cuttings. Careful consideration of these factors is critical in promoting successful propagation of *R. damascena* cuttings and other plant species. Further research is needed to understand the underlying mechanisms fully and develop optimal propagation protocols.

Number and length of roots

The contemporary study investigated the effects of the presence or absence of leaves and the dosage of auxin on the number and length of roots in *R. damascena* cuttings. The statistical analysis showed a significant interaction between these factors. The results revealed that the highest number and length of roots were observed in leafless cuttings treated with 10000 ppm of auxin. These cuttings exhibited 12 roots per cutting and a length of approximately 6 cm. In contrast, control cuttings treated with 0 ppm of auxin, regardless of the presence of leaves, had fewer than 2 roots per cutting and lengths of no more than 1 cm.

Interestingly, leafy cuttings treated with 1000 ppm of auxin showed longer roots than leafless cuttings treated with the same dose of auxin. Specifically, leafy cuttings treated with 1000 ppm of auxin had 7 roots per cutting with a length of 4.5 cm, while leafless cuttings treated with the same dose of auxin had 5 roots per cutting with a length of 2.5 cm. Although leafless cuttings treated with 10000 ppm of auxin showed the highest number and length of roots, the mortality rate with this treatment was significant. On the other hand, applying 10000 ppm of auxin to leafy cuttings resulted in a decrease in the number and length of roots compared to leafless cuttings treated with the same dose of auxin (Fig. 3).

The results of the present study provide valuable insights into the effects of the presence or absence of leaves and the dosage of auxin on the rooting ability of *R. damascena* cuttings. The statistical analysis revealed a significant interaction between these factors, indicating that the presence or absence of leaves and the dosage of auxin have a combined effect on the number and length of roots in cuttings. The finding that leafless cuttings treated with 10000 ppm of auxin exhibited the highest number and length of roots is consistent with previous studies that have reported that high concentrations of auxin can promote root formation in cuttings (26). However, the high mortality rate observed with this treatment suggests that it may not be a practical approach for promoting root growth in *R. damascena* cuttings. On the other hand, leafy cuttings treated with 1000 ppm of auxin showed a higher number and length of roots compared to leafless cuttings treated with the same dose of auxin, indicating that the presence of leaves can have a positive effect on rooting in cuttings. This aligns with previous studies that have reported that leaves can provide a source of carbohydrates and hormones that can stimulate root growth in cuttings (27).

In conclusion, the results of this study highlight the importance of considering both the presence of leaves and the dosage of auxin in promoting root growth in *R. damascena* cuttings. The findings suggest that a moderate concentration of auxin (such as 1000 ppm) may be more effective in promoting root growth in leafy cuttings. A higher concentration of auxin (such as 10000 ppm) may be more effective in promoting root growth in leafless cuttings. Further research is needed to determine the underlying mechanisms of these effects and to optimize the use of auxin in promoting rooting in plant cuttings.

Number of shoots newly formed

The present study investigated the effect of the presence or absence of leaves and the dosage of auxin on the formation of new shoots in *R. damascena* cuttings. The statistical analysis revealed a highly significant interaction between these factors regarding the number of newly formed shoots. Interestingly, the presence of leaves enhanced the ability of the cuttings to form new shoots compared to the leafless cuttings, both in the absence of auxin treatment and at moderate doses (1000 ppm) of auxin. Specifically, leafy cuttings formed 2.4 shoots per cutting without auxin treatment, whereas leafless cuttings formed only 1.5 shoots per cutting. Similarly, at 1000 ppm of auxin, leafy cuttings formed 3.2 shoots per cutting compared to 2.5 shoots per cutting in leafless cuttings. However, the effect of the presence of leaves on the formation of new shoots was reversed at higher doses (10000 ppm) of auxin. The leafless cuttings group treated with 10000 ppm of auxin exhibited the highest number of buds, with more than four shoots produced per cutting. In contrast, cuttings with leaves treated with the same auxin dose exhibited less performance, with less than 2 buds produced per cutting (Fig. 4).

The present study demonstrated the significant interaction between the presence or absence of leaves and the dosage of auxin on the formation of new shoots in *R. damascena* cuttings. The results indicated that the presence of leaves enhanced the ability of the cuttings to form new shoots, both in the absence of auxin treatment and at moderate doses (1000 ppm) of auxin. This finding is supported by previous studies that reported the positive

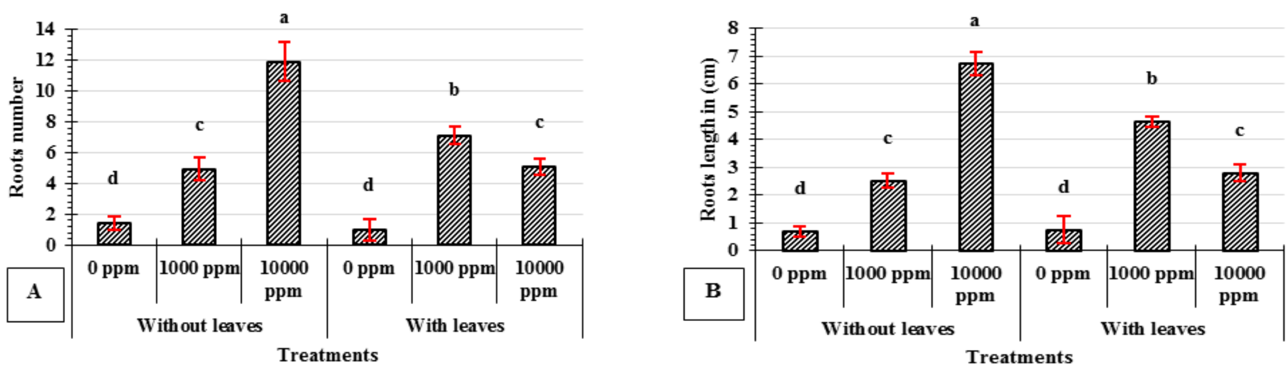

Fig. 3. Number and length of roots of cuttings induced by different treatments. The figure on left present the number of roots emerged and the figure on right represent the roots length in (cm). Means followed by the same letter are not statistically different at p < 5% according to the Tukey test.

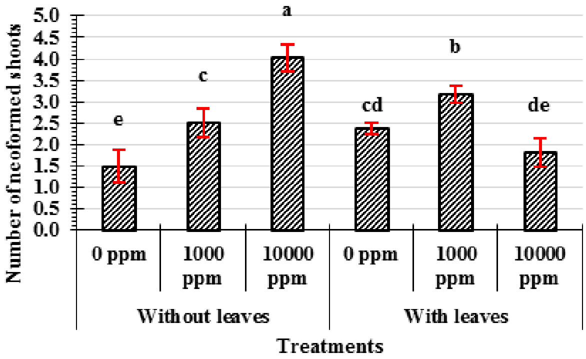

Fig. 4. Number of neoformed shoots induced by different treatments. Means followed by the same letter are not statistically different at p < 5% according to the Tukey test.

effect of leaves on the growth and development of cuttings in various plant species, including roses (28, 29). For example, investigation had been contributed the effects of leaf removal on the rooting and growth of cuttings and reported that the presence of leaves enhanced the rooting ability and the growth of the cuttings (30). Another study reported that leaves improved the rooting and growth of *Rosa centifolia* cuttings (31). The recent exploration also showed that the effect of the presence of leaves on the formation of new shoots was reversed at higher doses (10000 ppm) of auxin. This finding is consistent with the results of previous studies that reported the negative effect of high doses of auxin on the growth and development of cuttings. For example, another study revealed that the effect of different concentrations of IBA on the rooting and growth of cuttings and reported that high doses of IBA (5000 and 10000 ppm) inhibited the rooting and growth of the cuttings (32). Overall, the present study provides evidence for the significant interaction between the presence or absence of leaves and the dosage of auxin on the formation of new shoots in *R. damascena* cuttings. The results suggest that the presence of leaves can enhance the ability of the cuttings to form new shoots, especially at moderate doses of auxin. Still, this effect is reversed at higher doses of auxin. These findings have implications for the propagation of roses through cuttings and highlight the importance of optimizing the dose of auxin and the presence or absence of leaves for successful propagation.

Number of leaves per bud and number of leaflets per leaf

According to the results, the presence or absence of leaves on cuttings does not affect the number of leaves per bud when no auxin treatment is administered. However, during moderate auxin treatment (1000 ppm), the presence of leaves on cuttings has a positive impact. It significantly increases the number of leaves per bud (3 vs. 4.2 leaves per bud) compared to leafless cuttings. On the other hand, the effect is reversed when a high dose of auxin (10000 ppm) is applied, with leafless cuttings showing a significantly higher number of leaves per bud (4.5 vs. 1.7 leaves per bud) compared to leafy cuttings (Fig. 5A).

Conversely, the present study found that the number of leaflets per leaf was consistent across all treatments except for one particular case. It was observed that when applying a high dose of 10000 ppm auxin to leafless cuttings, the number of leaflets decreased from approximately 7 to 5.5 per leaf. This result suggests that the presence or absence of leaves may not significantly affect the number of leaflets per leaf in *R. damascena* cuttings. Still, the dose of auxin administered can harm this parameter (Fig. 5B).

The results of the present study indicate that the presence or absence of leaves on cuttings can significantly impact the number of leaves per bud depending on the dose of auxin administered. The positive effect of leaves on the number of leaves per bud during moderate auxin treatment (1000 ppm) is supported by previous studies that have reported the positive influence of leaves on the growth and development of cuttings in various plant species, including roses (33). This effect is likely due to the photosynthetic activity of the leaves, which can provide the necessary energy to form new leaves (34). On the other hand, the negative effect of leaves on the number of leaves per bud during high auxin treatment (10000 ppm) is consistent with previous studies that have reported the negative impact of high doses of auxin on the growth and development of cuttings. In addition, high doses of auxin can lead to hormonal imbalance and cause abnormalities in the growth and development of the cuttings (35).

The present study found that the number of leaflets per leaf was consistent across all treatments, except for

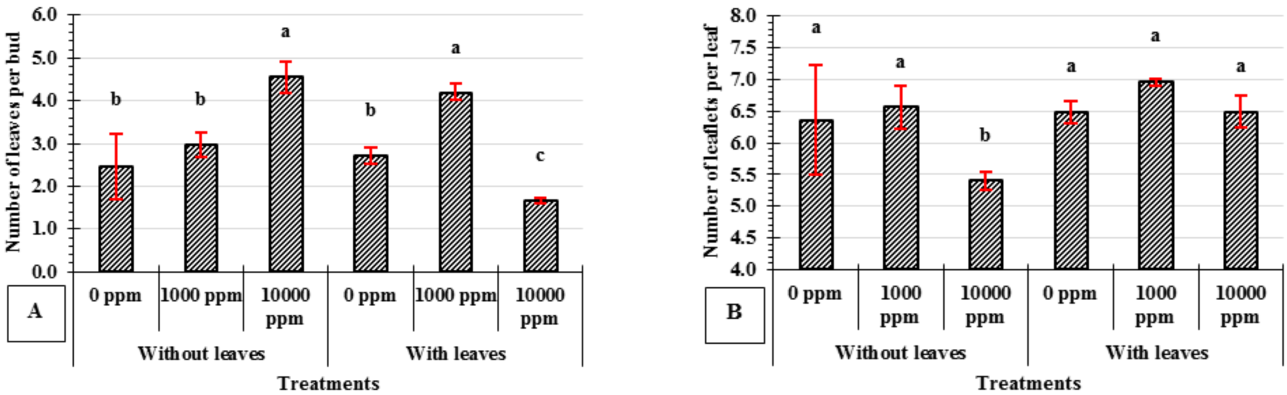

Fig. 5. Number of leaves per bud and of leaflets per leave of cuttings induced by different treatments. The figure on left present the number leaves emerged by bud and the figure on right represents the Number of leaflets per leave. Means followed by the same letter are not statistically different at p < 5% according to the Tukey test.

leafless cuttings treated with 10000 ppm of auxin, which showed a decrease in the number of leaflets per leaf. This result is supported by previous studies that have reported the negative impact of high doses of auxin on leaf development in various plant species (36). High doses of auxin can likely disrupt the normal developmental processes of leaves and lead to a decrease in the number of leaflets per leaf.

Root volume

The study results showed that the root volume was significantly influenced by the different auxin treatments, with no significant interaction observed between the dose and the presence or absence of leaves in the case of no auxin treatment or moderate auxin treatment (1000 ppm). However, with a dose of 10000 ppm, a highly significant difference was observed between leafy and non-leafy cuttings. Leafless cuttings treated with 10000 ppm of auxin had a prolific root system with a root volume of 14 cm^3 per cutting. In contrast, leafy cuttings treated with the same dose had a significantly lower maximum volume of 6 cm^3. These results are consistent with the findings of the study on the number and length of roots, where leafless cuttings treated with 10000 ppm of auxin had a significantly higher number and length of roots compared to leafy cuttings (Fig. 6).

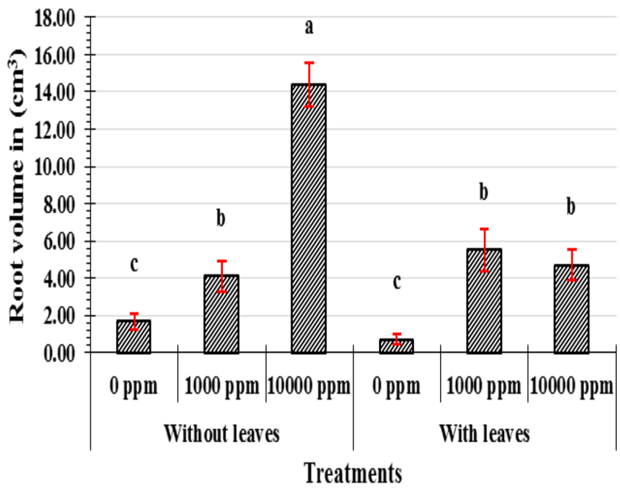

Fig. 6. Roots Volume induced by different treatments in (cm^3). Means followed by the same letter are not statistically different at $p < 5\%$ according to the Tukey test.

The results suggest that leaves on cuttings may inhibit the formation of roots in response to high levels of auxin. This is supported by previous studies, which have shown that the presence of leaves can limit the amount of auxin that reaches the base of the cutting, where root formation occurs (37). In addition, it has been suggested that the presence of leaves can also divert resources away from root formation towards shoot growth (38). On the other hand, the results also indicate that moderate levels of auxin (1000 ppm) can have a positive effect on root formation in both leafy and non-leafy cuttings. This may be because the lower concentration of auxin is more easily transported to the base of the cutting, where it can promote root formation.

Absorption and transpiration capacity of roots

Absorption speed

The initial 2 hrs of the uptake test revealed that leafy cuttings without auxin treatment had the highest average uptake rate, reaching a maximum of 0.8 g/h (Fig. 7), which was significantly higher than non-leafy cuttings with a maximum of 0.32 g/h uptake rate without auxin treatment. Similarly, leafy cuttings treated with auxin exhibited a much higher uptake rate than the auxin-treated non-leafy cuttings. However, it appears that auxin treatment reduced the uptake rate of formed roots in leafy and non-leafy cuttings, with higher auxin doses resulting in lower absorption capacities.

These results suggest that the presence of leaves plays a significant role in the uptake of water and nutrients in *R. damascena* cuttings, as leafy cuttings exhibited higher uptake rates than non-leafy cuttings. Additionally, the negative effect of auxin on the uptake rate suggests that the hormone may inhibit the development or functionality of the root system, particularly at higher doses. These findings are consistent with previous studies on the effect of auxin on root development in other plant species (39). However, further studies are needed to elucidate the underlying mechanisms of these effects in *R. damascena* and to optimize auxin application protocols for the propagation and cultivation of this important plant. Moreover, these results may have practical implications for the propagation and cultivation of *R. damascena*. For example, the presence of leaves during the cutting stage may increase the success rate of propagation by

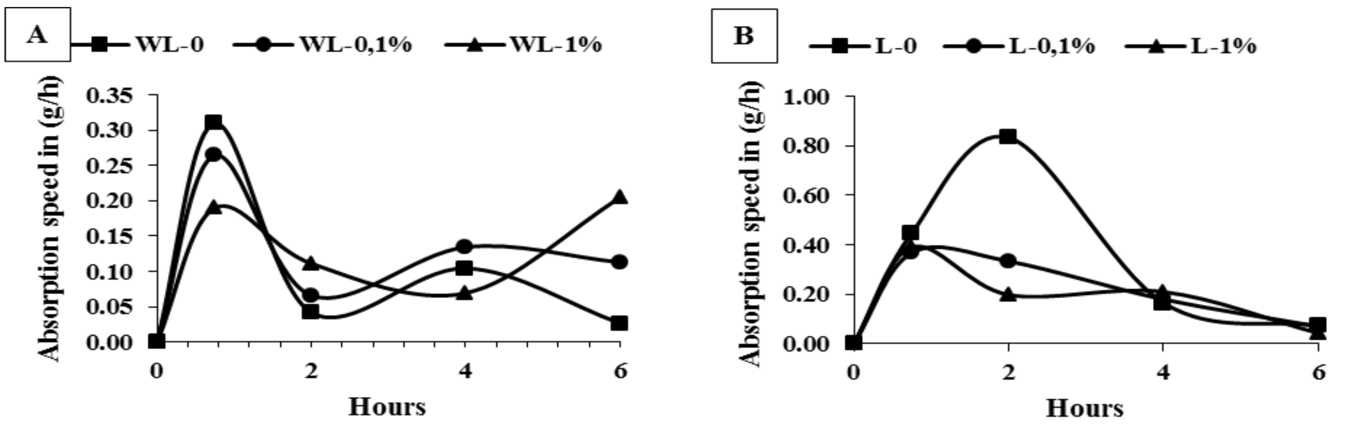

Fig. 7. Absorption rate, A: absorption rate of non-leafy cuttings (WL), B: absorption rate of leafy cuttings (L).

enhancing the uptake of water and nutrients, which is critical for establishing new plants (40). Additionally, careful consideration of the optimal dose and timing of auxin application may be necessary to avoid negative effects on root development and to ensure the efficient use of this growth regulator in *R. damascena* cultivation.

Transpiration speed

The results showed that leafy cuttings treated with 0.1% auxin and those without auxin treatment had transpiration rates of up to 0.6 g/h and 0.9 g/h respectively. In contrast, foliated cuttings transpired less, with a maximum of 0.15 g/h per treatment. Interestingly, un-foliated cuttings treated with 1% auxin had a maximum transpiration rate of 0.7 g/h, while foliated cuttings treated with the same dose had a maximum transpiration rate of 0.3 g/h (Fig. 8). The findings indicate that leaves play a substantial role in influencing the transpiration rate of *R. damascena* cuttings, and the effect can be further influenced by auxin treatment, potentially through its impact on root development and water uptake. However, further investigation is required to fully comprehend the underlying mechanisms and to optimize the application of auxin for the cultivation of *R. damascena*.

The results of the study suggest that both the presence of leaves and auxin treatment can significantly affect the transpiration rate in *R. damascena* cuttings. The observed decrease in transpiration rate in foliated cuttings compared to un-foliated cuttings without auxin treatment suggests that leaves regulate water loss through transpiration (41). However, the effect of leaves on transpiration rate was less pronounced in cuttings treated with auxin, possibly due to the influence of auxin on root development and water uptake, which could compensate for the impact of leaves on transpiration rate (42). Moreover, the finding that un-foliated cuttings treated with 1% auxin had a higher transpiration rate than foliated cuttings treated with the same dose suggests that auxin may have a differential effect on transpiration rate depending on the presence or absence of leaves. This highlights the need for further research to elucidate the underlying mechanisms of these effects and to optimize the use of auxin in the cultivation of *R. damascena.*

Cumulative absorption and transpiration rate of Roots

This study monitored root absorption and plant transpiration continuously for 40 hrs. Results showed that under auxin treatment, non-leafy cuttings had an increase in root uptake rate and progressively absorbed more with increasing auxin dose. However, leafy cuttings exhibited the opposite response, with the root uptake rate decreasing with increasing auxin dose during the 40-hour trial. Interestingly, leafy cuttings still absorbed the same amount of nutrients cumulatively throughout the experiment, regardless of treatment. Furthermore, the same trend was observed for seedling transpiration throughout the experiment (Fig. 9, Fig. 10). These findings suggest that the response to auxin treatment in *R. damascena* cuttings may depend on the presence or absence of leaves. Further investigation is needed to understand the underlying mechanisms fully.

These findings have important implications for the propagation and cultivation *of R. damascena*, as they highlight the potential benefits and limitations of using auxin treatment to enhance root development and nutrient uptake. The decrease in root uptake rate observed in leafy cuttings subjected to high auxin doses suggests that the hormone may exert adverse effects on the functionality or growth of the root system in these plants (43). This could have implications for the overall health and productivity of the plant, as adequate nutrient uptake is essential for proper growth and development. Furthermore, the fact that leafy cuttings still absorbed the same amount of nutrients cumulatively throughout the experiment regardless of treatment suggests that there may be compensatory mechanisms at play in these plants. This could indicate that leafy cuttings are better able to adapt to changes in their environment and optimize their nutrient uptake even under stress or unfavorable conditions (44). Thus, the study highlights the importance of continuous monitoring to understand the effects of treatments on plant physiology. The observed trends in absorption and transpiration rates indicate the complex interplay between the various factors influencing plant growth and development, including hormones, root system development, and leaf physiology. Further research is needed to investigate the effects of auxin

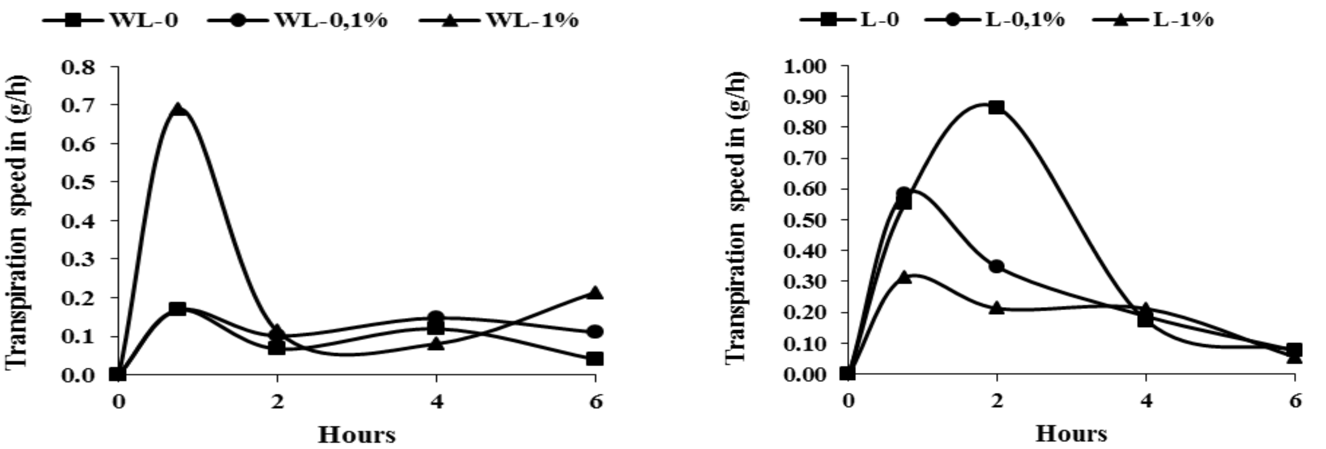

Fig. 8. Transpiration rate, A: transpiration rate of non-leafy cuttings (WL), B: transpiration rate of leafy cuttings (L).

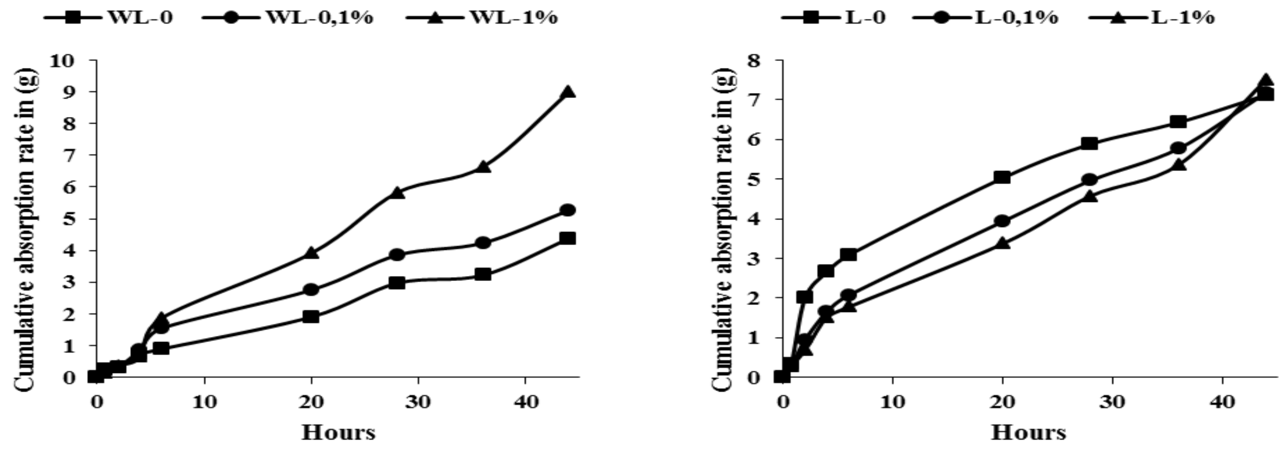

Fig. 9. Cumulative absorption rate, A: Cumulative absorption rate of non-leafy cuttings, B: Cumulative absorption rate of leafy cuttings.

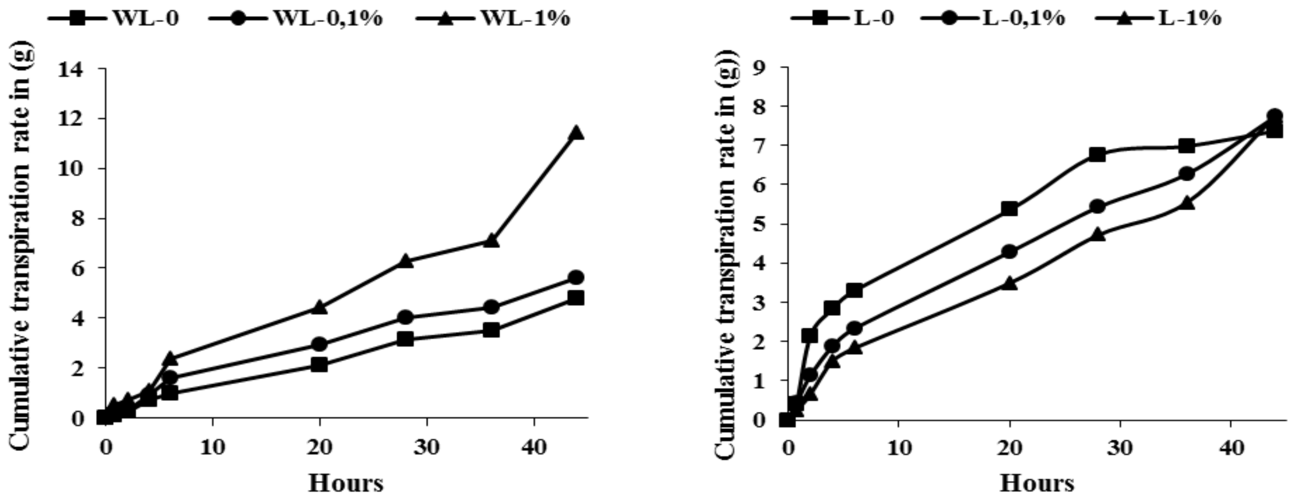

Fig. 10. Cumulative transpiration rate, A: Cumulative transpiration rate of non-leafy cuttings, B: Cumulative transpiration rate of leafy cuttings.

treatment on other aspects of plant physiology, such as photosynthesis and carbohydrate metabolism, to develop more effective propagation and cultivation strategies for *R. damascena*. Additionally, understanding the mechanisms behind the observed trends may have broader implications for cultivating and managing other plant species.

Leaf area

The results of this study suggest that the leaf surface area in *R. damascena* is significantly influenced by the type of cutting and the dose of auxin. Increasing the dose of auxin increased the leaf area, with a nearly 2-fold increase observed from 50 cm² to 98 cm² with an increase in auxin dose from 0 ppm to 10000 ppm in non-leafy cuttings. A similar trend was observed in leafy cuttings, but only when no auxin treatment was applied or when moderate doses of auxin (1000 ppm) were used. However, when a high dose of auxin (10000 ppm) was applied, the leaf surface area was significantly lower than in all other treatments. In addition, the study found that applying 1000 ppm of auxin to leafy cuttings resulted in the same leaf area as 10000 ppm in non-leafy cuttings. These findings suggest that auxin's effect on leaf area depends on both the dose of auxin and the presence or absence of leaves on the cutting (Fig. 11).

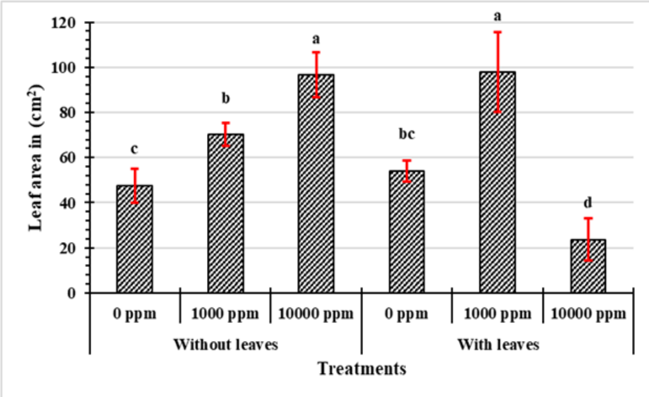

Fig. 11. The variation of vegetative surface according to different treatments; at 0 ppm,1000 ppm and 10000 ppm of IBA. Means followed by the same letter are not statistically different at p< 5% according to the Tukey test.

The observed increase in leaf area with increasing doses of auxin in *R. damascena* is consistent with previous studies on other plant species (45). For instance, a study on Arabidopsis thaliana found that applying exogenous auxin increased leaf size and promoted cell proliferation in the leaf primordia (46). Similarly, a study on pea plants showed that the application of auxin increased the rate of cell division and expansion in developing leaves (47). The finding that auxin's effect on leaf area depends on the presence or absence of leaves on the cutting is also supported by previous research (12). A study on

Conocarpus erectus cuttings showed that the presence of leaves on the cutting enhanced the rooting capacity and growth potential of the cuttings, which was attributed to the ability of the leaves to produce endogenous auxin (48). Another study on Pinus radiata cuttings found that needles on the cutting improved rooting and increased shoot and root growth, which was also attributed to the presence of endogenous auxin in the needles (49). Furthermore, the observed increase in leaf area with increasing doses of auxin in leafy cuttings only when no auxin treatment was applied or when moderate doses of auxin (1000 ppm) were used, could be explained by the fact that endogenous auxin levels are already high in leafy cuttings due to the presence of leaves. Therefore, the application of high doses of exogenous auxin may not significantly affect leaf area as the endogenous levels are already high.

Foliar analysis

Foliar analysis of macro and microelements of *R. damascena* plants was conducted to evaluate the effect of auxin dose and type of cuttings on nutrient assimilation, root development and leaf surface. The results were presented in the form of a heat map, which illustrated the content of the element in question by the density of its corresponding color. The analysis revealed that leaves emitted from leafless buds treated with 10000 ppm of auxin had the highest content of almost all elements compared to other treatments. The next highest content of elements was observed in leafy cuttings treated with 1000 ppm of auxin. All combinations studied accumulated a high average quantity of Cl and Na, except for the leafy cuttings treated with 0 or 10000 ppm of auxin, which had lower levels of the analyzed mineral elements. These findings indicate that auxin treatment can significantly impact the nutrient assimilation and mineral content of *R. damascena* plants and suggest that optimizing the use of auxin could improve the plant's overall health and growth. However, further studies are needed to fully understand the mechanisms underlying these effects (Fig. 12).

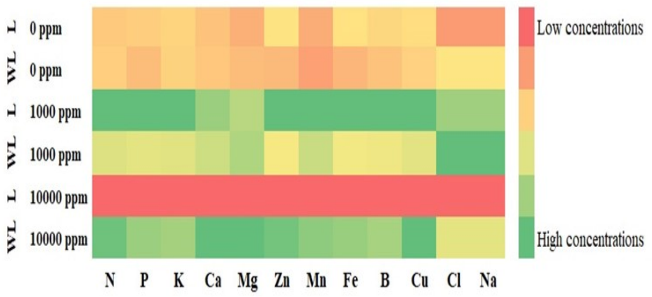

Fig. 12. Heat map representing dynamics of the nutrients as influenced by different treatment tested. The color gradient on the heat map legend indicated an ascending abundance of mineral compounds, with lower values observed from the top to the bottom end.

This finding is consistent with previous studies that have demonstrated the role of auxin in promoting root development and nutrient uptake in plants (50). Moreover, the high content of Cl and Na observed in all combinations studied, except for leafy cuttings treated with 0 or 10000 ppm of auxin, suggests that these minerals play a crucial role in the growth and development of *R. damascena.* However, the lower levels of these minerals observed in leafy cuttings treated with 0 or 10000 ppm of auxin suggest that the optimal dose of auxin needs to be determined to achieve the best nutrient assimilation and mineral content in the plant. The findings from this study suggest that optimizing the use of auxin could improve the overall health and growth of *R. damascena* plants. However, further studies are needed to fully understand the mechanisms underlying the observed effects and determine the optimal dose of auxin for different cuttings. Additionally, future studies could investigate the effect of other plant growth regulators on the growth and development of *R. damascena.*

Conclusion

Our investigation into the effects of auxin dose and cutting on *R. damascene mill.* has yielded significant insights into the physiological relationships between plant growth, nutrient assimilation, and survival rate of cuttings, with implications for the cultivation of this valuable plant species. Our findings indicate that non-leafy cuttings treated with high doses of auxin exhibited remarkable growth and nutrient assimilation but with a high mortality rate. In contrast, leafy cuttings treated with a moderate dose of auxin demonstrated a promising combination of positive results, amplifying rooting rate success and nutrient absorption efficiency. These findings underscore the importance of optimizing auxin application to enhance the cultivation of *R. damascena* by understanding the hub-and-spoke relationships that underlie plant physiology and offer valuable guidelines for sustainable cultivation methods for this historically and economically significant plant species, expanding the knowledge of the use of auxins and the importance of leaves in promoting successful rooting and nutrient absorption.

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Authors' contributions

Conceptualization: SEM and NS; Methodology: SEM, MM and LMIH; Software: SEM; Validation: HT, BZ, MM and LMIH; Formal analysis: SEM, ME and LMIH; Resources: SEM; Data curation: SEM, JZ and ME; Writing—original draft preparation: SEM and WM; Writing—review and editing: HT, WM, MM and LMIH; Supervision: MM and LMIH; Project administration: BZ, MM and LMIH. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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