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RESEARCH ARTICLE

In vitro rooting development and *ex vitro* acclimatization for *Rosa damascena* Mill. plant regeneration using auxin treatments

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Abstract

R. damascena, renowned for its aromatic essence, holds immense significance in various industries, including perfumery and cosmetics. However, its propagation presents challenges due to its recalcitrant nature. This study aimed to investigate micropropagation *in vitro* from single nodes as an alternative to traditional cutting methods, focusing on enhancing plant material preservation. Nodal explants were subjected to different auxin treatments (Indole-3-butyric-acid at T1:0.1, T2:0.5 and T3:1 mg/L; Indole-3-acetic-acid at T4:0.1, T5:0.5 and T6:1 mg/L; and 1-Naphthaleneacetic-acid at T7:0.5, T8:1 and T9:1.5 mg/L) to assess rooting efficiency and subsequent plant development. Results revealed a significant increase in rooting rate, with the highest rooting rate observed in the T3 treatment (97.22%) with 1 mg/L of IBA after 12 weeks of incubation. Moreover, the mortality rate varied significantly among treatments, with the highest rate observed in the control group (55.56%). The bud break rate was significantly higher in the T3 treatment compared to other treatments (100%). Correlations between morphological traits unveiled intricate relationships, highlighting the influence of auxin type and concentration on various parameters such as mortality and bud break rate. During the acclimatization process, a substrate composed of perlite, peat and sand in a ratio of 3:1:1 (v/v/v) was utilized. The IBA-treated plants demonstrated superior growth, with an average apical growth of (2.84 cm) and a leaf area of (20.95 cm^2) after 6 weeks. Our findings provide valuable insights into optimizing micropropagation techniques for *R. damascena*, thereby contributing to sustainable cultivation practices and meeting the increasing demand for this prized botanical resource.

Keywords

acclimatization; IAA; IBA; NAA; rooting induction; *R. damascena* Mill.

Introduction

Rosa damascena Mill., commonly known as Damask Rose, holds a prominent position botanically and industrially within the Rosaceae family. It is highly esteemed in agricultural and medical circles due to its diverse range of biochemical

compounds. The cultivation of this rose carries significant socio-economic importance on a global scale (1–3). In Morocco, the annual production of *R. damascena* is approximately 4000 tons, making it a key player in the country's essential oil industry (3). Currently, the propagation primarily occurs in nurseries through conventional methods like grafting and cuttings, driven by the increasing demand from various industries. However, these traditional techniques face significant challenges, such as inconsistencies and susceptibility to environmental factors, hindering their efficiency and reliability (4,5).

These limitations underscore the urgent necessity for alternative propagation methods that provide enhanced precision and control while mitigating conventional approaches' shortcomings. In response, *in vitro* micropropagation has arisen as a promising solution, ensuring both genetic integrity and meticulous management of rooting processes (6). By enabling entire plant generation from a single cultured node, this method boasts higher success rates and opens new avenues for the floral and horticultural industry (7). Nevertheless, *R. damascena in vitro* micropropagation unveils a continuum of obstacles and breakthroughs in managing this invaluable plant. While this technique shows promise, it still grapples with numerous impediments, including low culture viability and challenges in stimulating cell proliferation (8). Among these challenges, root induction remains a significant challenge in the micropropagation of *R. damascena*. While shoot proliferation can be easily achieved, *in vitro* rooting is often inefficient, hindering the successful acclimatization and ex vitro establishment of plantlets (6). This limited rooting competence can be attributed to complex factors, including genotype dependence, imbalanced phytohormone levels and inadequate nutrient supply in the culture medium (8).

The significance of auxins in the triumph of micropropagation, particularly in *R. damascena*, unfolds through their multifaceted influence on critical developmental stages, including rooting. Delving into the chemical, physiological and molecular impacts of auxins: Indole butyric acid (IBA) (9), α-naphthaleneacetic acid (NAA) (8) and indole-3-acetic acid (IAA) (10)-reveals their nuanced effects on pivotal micropropagation phases.

This study investigated the influence of different types of auxins (IBA, IAA, NAA) at various concentrations on *R. damascena* micropropagation, including rooting efficiency and ex vitro acclimatization. A systematic approach was employed to identify optimal auxin levels that promote robust *in vitro* growth through the manipulation of auxin-mediated processes. The use of high concentrations of NAA for rooting *R. damascena*, compared to lower concentrations of IBA and IAA, is due to several factors. *R. damascena* is more sensitive to high levels of NAA, while IBA and IAA promote rooting at lower concentrations. Additionally, NAA is often more effective at inducing the formation of adventitious roots, but its chemical stability sometimes requires higher doses. In contrast, IBA and IAA, being less phytotoxic, can act effectively at reduced levels. By elucidating the mechanisms underlying the auxin-micropropagation interplay, this research aims to provide valuable knowledge for refining *R. damascena* propagation protocols, potentially leading to significant advancements in cultivation practices and maximizing its industrial applications.

Materials and Methods

Selection of explant material

The explants utilized in this research originated from *R. damascena* obtained from an organic rose farm affiliated with the "Soffi" cooperative, situated in Kelâat M'Gouna (31.2428997° N,-6.1055540°S), Morocco. The shrubs ranged in height from 100 to 150 cm, with each shrub boasting 10 to 20 1-year stems, each of which carried approximately 20 to 25 axillary buds. Nodal explants, measuring around 1.5 cm in length and featuring a single axillary bud, were selected from the mid-stem region of 1-year stems. This specific stem segment was chosen due to its greater thickness, measuring between 0.4 and 0.6 cm in diameter.

Sterilization of explants

Nodal explants, measuring around 1.5 cm in length and featuring a single axillary bud, were selected from the mid-stem region of 1-year stems and washed primarily with running water and soap for 8 minutes to eliminate all dirt and debris. The micro -cuttings were used in this study and subjected to a sterilization protocol. They were first disinfected in a 70% ethanol solution (v/v) for 2 minutes, then rinsed in sterilized deionized water. The micro-cuttings were then immersed in a 30% bleach solution (NaOCl2) added with a few drops of Tween 20 (Polyethylene Sorbitan Monolaurate, Sigma-Aldrich) for 20 minutes and rinsed again three times for 15 minutes in sterilized deionized water. The tissues of rose bushes are very sensitive to a browning phenomenon due to polyphenols. To overcome this difficulty, the micro-cuttings were then soaked for 30 minutes in a sterile antioxidant solution containing 2 g/l of polyvinylpyrrolidone (PVP). The surface disinfection steps were conducted under a horizontal laminar hood. The sterilized micro-cuttings were finally dried on sterile absorbent paper.

Medium preparation

To assess the influence of auxin type and its concentration on the rooting process of *R. damascena*, micro-cuttings were carefully positioned in glass bottles containing 50 ml of MS medium. The cultural medium was prepared according to Murashige and Skoog (1962). It was solidified with 3.0 g/L phytagel (Sigma-Aldrich, St. Louis, USA) and its pH was adjusted to 5.8. The media were portioned into 500 ml jars, 50 ml in each and autoclaved for 20 min at 121°C and 1 kbar. This medium was supplemented with 30 g/l of sucrose and various concentrations of auxins were introduced: Indole-3-butyric acid (IBA) (0.1, 0.5, 1 mg/L), Indole-3-acetic acid (IAA) (0.1, 0.5, 1 mg/L) and 1-Naphthaleneacetic acid (NAA) (0.5, 1, 1.5 mg/L). The control medium, serving as the baseline, did not incorporate any auxins, making it the control treatment for the experiment.

In vitro rooting of R. damascena micro-cuttings

The basal part of the micro-cuttings was inserted in the rooting medium (MS+ auxin), while the apical part remained exposed to air. The micro-cuttings were cultured in a controlled environment for 12 weeks. The environmental conditions were optimized for the proliferation of *R. damascena*, maintaining a temperature range of 22-25 °C, relative humidity between 60-80%, a photoperiod of 16-8 days/nights and a photosynthetic photon flux of 50-100 µmol m-2 s-1. The experiment was conducted as a randomized design with 2 variables (Table 1),

using a total of 10 combinations, including control treatment. Each treatment consisted of 3 replicates and within each replicate, 12 micro-cuttings were composed. Following the first 8 -week period, the rooted micro-cuttings were subsequently transferred to the same medium employed previously to stimulate root development for 4 weeks. After twelve weeks, the following parameters were noted: mortality rate, rooting rate and rooting time, average number of roots formed per cutting and average root length, bud break rate and number of leaflets per leaf formed.

Acclimatation monitoring

For the acclimatization test, twelve-week-old *in vitro* plants with established roots were delicately removed from the glass bottles and their roots were thoroughly rinsed with distilled water to eliminate any residual culture medium. Subsequently, these *in vitro* plants were transplanted into 500 ml pots filled with a sterile mixture of perlite and coconut fiber (1:1, v/v). The pots were then positioned in a mini greenhouse covering an area of 12 m^2, enclosed with anti-condensation plastic. This controlled environment maintained a temperature range of 20 to 25 °C, with a photoperiod of 14 hours light and 10 hours dark, along with a relative humidity level between 80% and 100%.

Table 1. Treatments tested for explant rooting and shoot establishment.

Treatment	Auxin Type	Dose
T1	IBA	0.1 mg/L
T2	IBA	0.5 mg/L
T3	IBA	1 mg/L
T4	IAA	0.1 mg/L
T5	IAA	0.5 mg/L
T6	IAA	1 mg/L
T7	NAA	0.5 mg/L
T8	NAA	1 mg/L
T9	NAA	1.5 mg/L

After two weeks, the *in vitro* plants were transplanted into another 500 ml pots for enhanced acclimatization containing a mixture of perlite, peat and sand (3:1:1, v/v/v). These larger pots were arranged on a tide table, receiving 3/4 shade provided by an aluminum screen (Fig. 1), within a Richel-type greenhouse spanning an area of 50 m^2. The greenhouse hada cooling system, maintaining a temperature range of 25±3° C. Observations were made four weeks post-potting, measuring their height and their leaf area. Each treatment encompassed 5 replicates, totaling 50 plants across all treatments.

Laef area assessment

Leaf surface area was precisely quantified utilizing the "CANOPEO" software, a tool designed to accurately measure leaf area as a percentage with a predefined scale. Initially, the conversion from pixels to cm^2 was calculated. A photograph was taken of downward-facing leaves arranged on a black background. Subsequently, the image underwent analysis to determine the extent of the leaf surface area, quantified as the leaf area index (LAI).

Statistical analysis

The data was subjected to analysis of variance (ANOVA) using Minitab® statistical software version 20 (Minitab, Inc., State College, PA, USA). The confidence intervals were set at 95% and p values were used to show the level of statistical significance of the differences ($p \leq 0.05$: significant, $p \leq 0.01$: highly significant and $p < 0.001$: very highly significant). Tukey HSD and the Gupta post-hoc tests (parametric tests) were used to compare the means between the different treatments for datasets that are not normally distributed or displayed a heterogeneity of variances; a non-parametric Kruskal-Walli's test was used instead of the parametric tests. Furthermore, to visually compare the impact of the different treatments analyzed on various morphological traits, allowing easy assessment of their effects across multiple parameters in one view, a correlation analysis (Pearson) was used to examine relationships between all the variables.

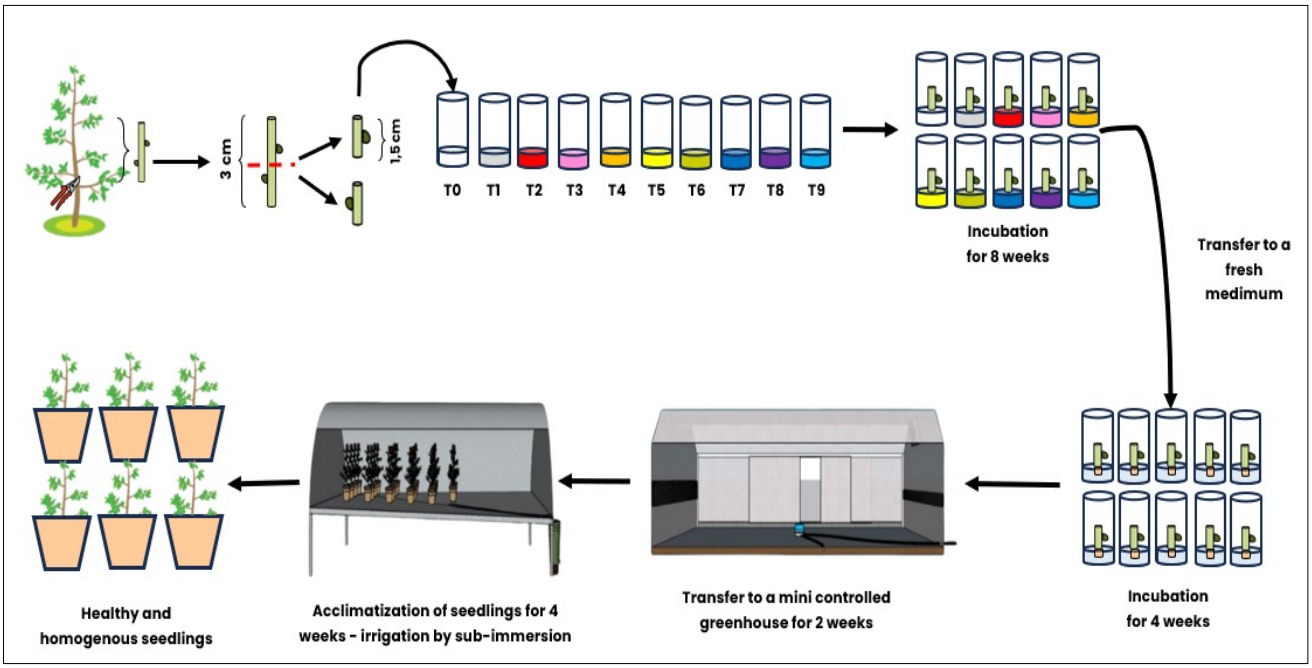

Fig.1. Schematic representation of the procedure used for *R. damascena in vitro* micropropagation. **T0:** Control medium (without auxin); **T1:** IBA (0.1 mg/L); **T2:** IBA (0.5 mg/L); **T3:** IBA (1 mg/L); **T4:** IAA (0.1 mg/L); **T5:** IAA (0.5 mg/L); **T6:** IAA (1 mg/L); **T7:** NAA (0.5 mg/L); **T8:** NAA (1 mg/L); **T9:** NAA (1.5 mg/L).

Results

Nodal explants, approximately 1.5 cm long and containing a single axillary bud, were sourced from mature plants cultivated in the field. These explants were then evaluated for root induction on MS media supplemented with varying concentrations of auxins, including IBA, IAA and NAA. The rooting phase commenced during the second week of cultivation (Fig. 2), reaching its peak between weeks 8 and 12. A significant increase in rooting percentage was observed over time. Micro cuttings treated with NAA exhibited early initiation of rooting as early as the second week. However, their rooting rate remained relatively low compared to other treatments, showing the lowest percentage of rooting. Conversely, micro cuttings treated with IAA showed a delayed onset of rooting starting from the fifth week, with a moderate rooting rate induced by this type of auxin. On the other hand, IBA initiated rooting as early as the third week, reaching a peak around the ninth week of monitoring. Particularly, treatment T3, associated with the highest dose of IBA, demonstrated a notable increase, from 15% in the third week to 97% in the twelfth week, indicating the highest rooting rate. All treatments showed a positive correlation with increasing auxin dose, highlighting that an increase in dose leads to a proportional increase in rooting rate.

After 12 weeks of cultivation, various parameters including mortality rate, rooting rate, number of emerged roots, root length, budding rate, number of shoots formed and number of leaflets per leaf were analyzed. A very highly significant effect of treatments was observed on the mortality rate as compared with control (F = 18.23; P = 0.000), except for treatments T7 and T8 of NAA auxin. The control plants exhibited the highest mortality rate (55.56 ± 9.62), followed by those in the T7 (44.44 ± 4.81) and T8 (36.11 ± 4.81) treatments. No significant effect was noted between treatments T1 and T2. Whatever the treatments, the analysis showed a significant difference between the different treatments and the control. The rooting rate showed a very highly significant response to treatments (F = 34.82; P = 0.000). The results presented in Table 2 clearly show that T3 treatment of auxin IBA resulted in the highest number of roots per explant (97.22 ± 4.81), which was significantly higher than the control with an average of 13.89 ± 4.81 roots per explant and the highest of all other treatments.

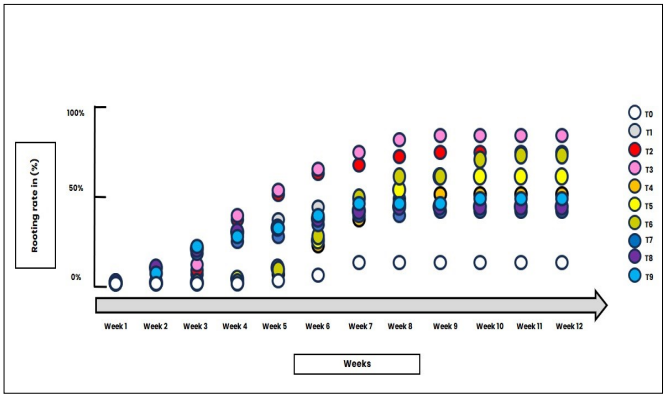

Fig.2. Evolution of rooting time of the micro-cutting. **T0:** Control medium (without auxin); **T1:** IBA (0.1 mg/L); **T2:** IBA (0.5 mg/L); **T3:** IBA (1 mg/L); **T4:** IAA (0.1 mg/L); **T5:** IAA (0.5 mg/L); **T6:** IAA (1 mg/L); **T7:** NAA (0.5 mg/L); **T8:** NAA (1 mg/L); **T9:** NAA (1.5 mg/L).

Additionally, the number of emerged roots per plant and root length were highly significantly influenced by treatments (F = 134.98; P = 0.000) and (F = 123.38; P = 0.000), respectively. The greatest number of emerged roots per explant (3.09±0.08) and the greatest root length (34.25 ± 0.16) cm were obtained when the medium was incorporated with 1 mg L-1 of IBA (Table 2), almost double that of the control treatment. Fig. 3 below shows the effects of various concentrations of the different auxins analysed on root formation and growth.

The treatments applied had a highly significant effect on the bud break rate (F = 9.69; P = 0.000). The treatment T3 showed a notably higher rate compared to other treatments and the control. Similarly, the number of shoots that emerged was highly significantly affected by the treatments (F = 4.75; P = 0.002), with the control group having a higher number of emerged shoots compared to the treated ones. All treatments also significantly impacted the number of leaflets per leaf (F = 32.09; P = 0.000). The treatments T1, T2 and T6 did not differ considerably in leaflet number, but they were notably higher than other treatments and lower than the control (5.33 ± 1.16).

Pearson correlation analysis unveils intricate relationships among various morphological traits, revealing significant associations and providing valuable insights into plant development dynamics. The correlation analysis between morphological traits, depicted in Fig. 4, highlights a very highly significant negative correlation among rooting rate, bud break rate and mortality rate (r = -0.88; p ≤ 0.001, r = -0.95; p ≤ 0.001, respectively), indicating a strong inverse relationship between

Table 2. Impact of different Treatments on mortality rate, rooting rate, number of emerged roots, roots length in mm, bud break rate, number of forms shoots and number of leaflets per leaf, of *R. damascena* micro-cuttings, after 12 weeks of incubation. **T0:** Control medium (without auxin); **T1:** IBA (0.1 mg/L); **T2:** IBA (0.5 mg/L); **T3:** IBA (1 mg/L); **T4:** IAA (0.1 mg/L); **T5:** IAA (0.5 mg/L); **T6:** IAA (1 mg/L); **T7:** NAA (0.5 mg/L); **T8:** NAA (1 mg/L); **T9:** NAA (1.5 mg/L). Means followed by the same letter are not statistically different at p<5% according to the Tukey test.

		Roots				Shoots	
Treatment	**Mortality rate**	**Rooting rate**	**Number of emerged roots**	**Roots length (mm)**	**Bud break rate**	**Number of shoots formed**	**Number of leaflets per leaf**
T0	55.56% ± 9.62%^{a}	13.89% ± 4.81%^{e}	1.50 ± 0.50^{e}	22.17 ± 2.57^{de}	44.44% ± 9.62%^{c}	1.75 ± 0.25^{a}	5.33 ± 1.16^{a}
T1	19.44% ± 9.62%^{cde}	58.33% ± 8.33%^{cd}	2.34 ± 0.09^{d}	19.89 ± 0.19^{ef}	80.56% ± 9.62%^{ab}	1.34 ± 0.20^{ab}	3.05 ± 0.25^{bc}
T2	11.11% ± 4.81%^{de}	86.11% ± 4.81%^{ab}	3.03 ± 0.06^{c}	27.53 ± 0.47^{b}	88.89% ± 4.81%^{ab}	1.06 ± 0.11^{b}	3.72 ± 0.17^{b}
T3	0.00% ± 0.00%^{e}	97.22% ± 4.81%^{a}	3.09 ± 0.08^{c}	34.25 ± 0.16^{a}	100.00% ± 0.00%^{a}	1.03 ± 0.05^{b}	1.89 ± 0.10^{cde}
T4	22.22% ± 9.62%^{cd}	58.33% ± 8.33%^{cd}	1.76 ± 0.10^{e}	14.54 ± 0.26^{g}	72.20% ± 19.20%^{abc}	1.38 ± 0.24^{ab}	1.18 ± 0.16^{de}
T5	13.89% ± 4.81%^{de}	69.44% ± 4.81%^{bc}	1.80 ± 0.15^{de}	16.49 ± 0.15^{g}	86.11% ± 4.81%^{ab}	1.42 ± 0.19^{ab}	0.57 ± 0.38^{e}
T6	13.89% ± 4.81%^{de}	83.33% ± 8.33%^{ab}	1.90 ± 0.11^{de}	23.76 ± 0.16^{cd}	94.44% ± 4.81%^{a}	1.05 ± 0.18^{b}	3.66 ± 0.49^{b}
T7	44.44% ± 4.81%^{ab}	47.22% ± 4.81%^{d}	3.48 ± 0.27^{c}	19.40 ± 0.71^{f}	47.22% ± 4.81%^{c}	1.23 ± 0.21^{ab}	1.04 ± 0.21^{de}
T8	36.11% ± 4.81%^{abc}	50.00% ± 8.33%^{cd}	4.22 ± 0.06^{b}	21.61 ± 0.63^{def}	58.33% ± 14.43%^{bc}	1.47 ± 0.17^{ab}	1.64 ± 0.36^{de}
T9	25.00% ± 8.33%^{bcd}	55.56% ± 9.62%^{cd}	5.75 ± 0.08^{a}	25.83 ± 0.17^{bc}	69.40% ± 17.30%^{abc}	1.48 ± 0.16^{ab}	1.91 ± 0.27^{cd}

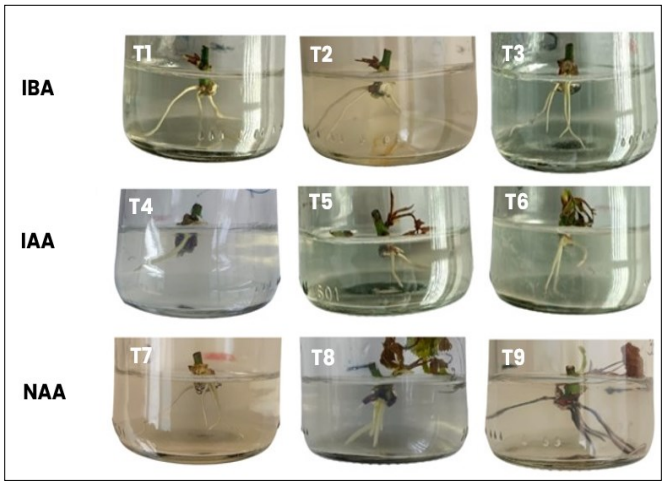

Fig.3. Micropropagation of *R. damascena* grown on MS medium supplemented with different auxin treatments IBA, IAA and NAA. IBA (**T1**:(0.1 mg/L) ; **T2:** (0.5 mg/L) ; **T3**:(1 mg/L)) ; IAA (**T4**:(0.1 mg/L) ; **T5**:(0.5 mg/L) ; **T6**:(1 mg/L) ; NAA (**T7**:(0.5 mg/L) ; **T8**:(1 mg/L) ; **T9**:(1.5 mg/L)).

these variables. Additionally, a significant negative correlation was found between root length and mortality rate (r = -0.40; p ≤ 0.05). Moreover, a highly positive significant correlation was observed between the number of shoots formed and mortality rate (r = 0.5; p ≤ 0.01). Furthermore, a very highly significant positive correlation was found between rooting rate and bud break rate (r = 0.84; p ≤ 0.001) and a highly significant correlation was observed between rooting rate and root length (r = 0.47; p ≤ 0.01). Conversely, a very highly significant negative correlation was observed between rooting rate and the number of shoots formed (r = -0.78; p ≤ 0.001). Additionally, a positive significant correlation was noted between the number of emerged roots and root length (r = 0.38; p ≤ 0.05). Similarly, a significant positive correlation was observed between bud break rate and root length (r = 0.38; p ≤ 0.05), while a significant negative correlation was found between the number of emerged roots and root length (r = -0.37; p ≤ 0.05). Finally, a negative significant correlation was observed between bud break rate and the number of shoots formed (r = -0.42; p ≤ 0.05).

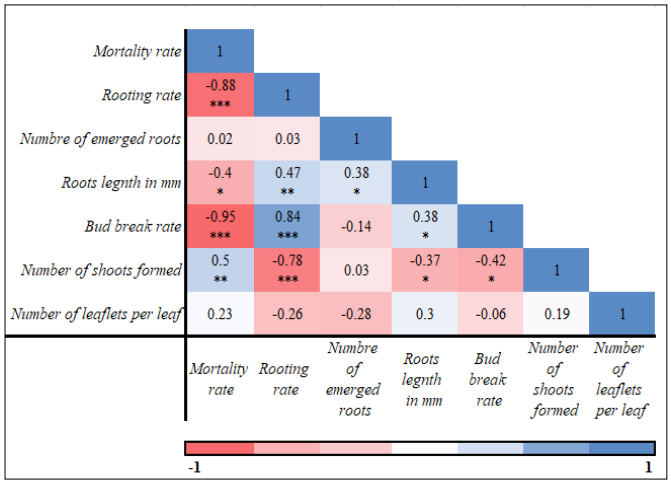

Fig.4. Pearson's correlation matrix for all the investigated morphological traits. Values in the matrix represent Pearson's correlation coefficient. Asterisks *, ** and *** correspond to the following significance levels: p < 0.05, p< 0.01 and P< 0.001, respectively.

During acclimatization, *in vitro* rooted plantlets were acclimatized for 6 weeks after being washed with sterile distilled water and treated. Subsequently, their apical growth and leaf area were evaluated. The treatments had a very highly significant effect on these two parameters (F=6.22; P=0.000, F=44.18; P=0.000) respectively. No significant differences were observed between plants from different treatments and the control group. However, significant variations were noted in apical length among the different auxin treatments. Specifically, apical length was higher in the IBA auxin treatments compared to those with IAA and NAA auxins. Moreover, treatment T1 exhibited significantly greater apical length compared to all other treatments and the control with an apical growth of 2.84 ± 0.63 cm. Regarding leaf area, treatment T1 demonstrated the highest value (20.95 ± 3.40), followed by T2 (15.29 ± 2.71) and the lowest was observed in T3 (13.90 ± 0.27) using the IBA auxin (Table 3).

Table 3. Impact of different treatments on plantlets obtained after 6 weeks of acclimatization. **T0:** Control medium (without auxin); **T1:** IBA (0.1 mg/L); **T2:** IBA (0.5 mg/L); **T3:** IBA (1 mg/L); **T4:** IAA (0.1 mg/L); **T5:** IAA (0.5 mg/L); **T6:** IAA (1 mg/L); **T7:** NAA (0.5 mg/L); **T8:** NAA (1 mg/L); **T9:** NAA (1.5 mg/L). Means followed by the same letter are not statistically different at p<5% according to the Tukey test.

Treatment	Apical growth (cm)	Leaf area (cm3)
T0	1.70 ± 0.50^{ab}	9.24 ± 1.12^{cd}
T1	2.84 ± 0.63^{a}	20.95 ± 3.40^{a}
T2	2.82 ± 0.94^{a}	15.29 ± 2.71^{b}
T3	2.80 ± 0.89^{a}	13.90 ± 1.27^{b}
T4	1.34 ± 0.74^{b}	8.26 ± 1.08^{d}
T5	1.38 ± 0.38^{b}	5.82 ± 0.82^{de}
T6	1.36 ± 0.62^{b}	3.59 ± 1.41^{e}
T7	0.86 ± 0.79^{b}	12.38 ± 1.74^{bc}
T8	1.54 ± 0.61^{ab}	4.38 ± 0.71^{e}
T9	1.20 ± 0.31^{b}	8.54 ± 2.01^{cd}

Discussion

Micropropagation stands out as an alluring method, offering precise control and efficiency in cultivating plants within a controlled, sterile environment (11,12). Within this setting, auxins play a pivotal role, guiding essential stages of the process (13). Additionally, auxins propel root development in micro cuttings, a pivotal phase in establishing self-sustaining plants (9,14). Beyond these initial stages, auxins facilitate the acclimatization process, aiding the transition of micro propagated plants to non-sterile conditions, ensuring their adaptation and survival (15). The intricate interplay between auxins and various micropropagation phases underscores their indispensable role in orchestrating the complex events vital for successful plant multiplication in controlled environments (16).

Different auxins, such as (IAA), (IBA) and (NAA), exert unique effects on *R. damascena* rooting. Our experiment on micro cuttings revealed specific responses to each auxin, influencing rooting dynamics and overall plant growth. NAA-treated micro cuttings showed early rooting initiation but with limited rooting rates, possibly due to hormonal imbalances. Conversely, IAA-treated micro cuttings displayed delayed rooting onset but sustained root primordia formation. Interestingly, IBA-treated micro cuttings exhibited early and robust rooting, particularly in higher concentrations, emphasizing the importance of auxin concentration in rooting dynamics. Understanding these differential effects could refine propagation strategies for *R. damascena*, enhancing commercial production.

The results of our study demonstrated that the micro cuttings treated with IBA exhibited superior rooting and shoot development. This was evidenced by a higher rate of bud break,

an increased number of developed shoots and a greater number of leaflets per leaf. This result was also observed with other authors; the rooting rate attends 70% depending on the origin of cuttings and the applied concentration of IBA (17). Otherwise, a higher rooting rate between (88.27% and 89.8%) than that obtained in our study was obtained in the study of Tharwat et al (18).

These findings ensured robust aerial growth, leading to enhanced leaf surface area and photosynthesis. This suggests that IBA plays a role in modulating auxin and cytokinin levels, thereby optimizing plant growth (19). According to several authors, the IBA emerges as a fundamental player in *R. damascena* micropropagation (9,11). It acts as the primary catalyst initiating the sequence for successful micropropagation (20). IBA intricately manipulates auxin signalling pathways, triggering the upregulation of crucial genes essential for cell wall expansion (21). This orchestrated activity paves the way for a robust rooting system, laying the groundwork for further growth and development. Investigating IBA treatment revealed significant alterations in rooting and shoot development, possibly mediated through auxin signalling pathways. Further research should delve into the underlying mechanisms and optimize IBA concentrations for efficient micropropagation.

In parallel, the results of our study have demonstrated that the application of a high dose of IAA ensures a favourable rooting rate of plants reaching 83.33%. Our results are consistent with those obtained by Monteuuis and Bon who demonstrated that treatment with IAA gives the best results for acacia rooting (22).

These observations align perfectly with the findings of Pati et al. (23,24), indicating that IAA meticulously regulates genes associated with root meristematic activity and elongation in the micropropagation of *R. damascena*. This phytohormone lays the foundations for a well-established root system, essential for the survival of seedlings (25). This phytohormone lays the foundation for a well-established root system, pivotal for plantlet survival (25). Additionally, (IAA) emerges as a critical regulator, modulating the delicate interplay between auxin and abscisic acid (ABA) signalling pathways (26). Furthermore, IAA's role in regulating water balance, stress responses and stomatal conductance proves vital in facilitating plantlet adaptation to changing environmental conditions (27,28). IAA treatment influences various aspects of *R. damascena* development, including root, shoot and leaf morphology (7). Despite a delayed rooting onset, IAA-treated micro-cuttings displayed moderate rooting rates, highlighting its efficacy in root growth promotion (29). Additionally, IAA treatment positively influenced shoot development, suggesting its multifaceted impact (30). However, during acclimatization, IAA's effects were nuanced, warranting further investigation. Indeed, on semi-woody cuttings of jojoba (Simmondsia chinensis L.), it has been demonstrated that among the three auxins tested (AIB, AIA and ANA), it is AIB that gives the best results, followed by AIA, then ANA (31).

α-naphthaleneacetic acid proves vital in enhancing roots formation during *R. damascena* micropropagation (32). Its role primarily involves boosting roots growth and compactness, augmenting the overall effectiveness of roots formation (33). Furthermore, NAA significantly contributes to root meristem expansion, ensuring the establishment of a well-structured and efficient root system crucial for successful plantlet establishment (34). Exploring NAA's effects unveils its intricate mechanisms in plant development, particularly in rooting and overall health (35). NAA likely initiates rooting through complex signalling pathways involving Auxin Response Factor (ARF) proteins, facilitating root development gene expression (36). NAA is known to accelerate early root development, which is consistent with our observation of rooting commencing around the second week. However, this relatively early initiation might be followed by a slower progression, suggesting a potentially transient signalling effect of NAA. This transient effect could limit NAA's ability to promote optimal overall rooting rates. Our findings support this hypothesis, as NAA treatment resulted in the lowest minimum rooting rate compared to other tested auxins (37). Nevertheless, NAA treatment improves plant health, reducing mortality rates and promoting shoot development (38). Further research should optimize NAA concentration for balanced root initiation and investigate its interactions for improved acclimatization success.

In the intricate world of auxin signalling, the interplay between NAA, IAA and IBA unveils a multifaceted landscape of rooting induction and bud break facilitation, with each auxin type offering distinct physiological and molecular characteristics. NAA, characterized by its rapid initiation of cell division, harnesses specific auxin receptor pathways to swiftly activate genes pivotal for root initiation (39). This early spark, however, is dimmed by NAA's susceptibility to rapid metabolism or conjugation within the plant system, resulting in a transient auxin signal that may limit overall rooting rates (40). In contrast, IAA presents a more nuanced role, engaging in a complex network of metabolic conversions upon application. Initially, IAA exhibits a delay in rooting induction, a consequence of its propensity towards promoting shoot growth before reaching a tipping point that favours root development (41). This intricate balancing act between shoot and root promotion reflects the diverse metabolic pathways that IAA traverses within the plant, yielding metabolites with differential effects on root and shoot phenotypes (42). Enter IBA, emerging as a beacon of stability amidst the flux of auxin dynamics. Recent investigations illuminate IBA's resilience to rapid metabolic turnover, underscoring its capacity to sustain auxin signalling for prolonged periods compared to its counterparts (43). This stability is underpinned by the diminished susceptibility of IBA to enzymatic metabolism and conjugation, ensuring a more sustained and robust signal conducive to root development pathways (44). Moreover, the elucidation of specific enzymes involved in IBA metabolism holds promise for targeted manipulations aimed at further enhancing rooting efficiency, offering exciting prospects for refined auxin application strategies (45). Delving deeper into morphological traits unveils the intricate molecular mechanisms underpinning auxin-mediated responses. IBA emerges as a potent orchestrator of root development, activating genes crucial for root meristem activity and root hair formation (46,47). The stability of IBA's signal translates into a stronger and more sustained activation of these genes, fostering enhanced cell division and elongation within the root system (48). The optimal concentration of T3 IBA strikes a delicate balance between rooting induction and bud break facilitation, ensuring maximal efficacy in both processes while avoiding potential inhibitory effects associated with high auxin concentrations. During the acclimatization phase, while

initial auxin types imprint their influence on developmental processes, plants ultimately establish their hormonal equilibrium for sustained growth. However, the lingering effects of IBA manifest in superior apical length, underscoring its enduring impact on growth processes initiated during the rooting and establishment phases (49). Exploring gene expression patterns during acclimatization holds promise for unravelling the underlying mechanisms driving IBA's lingering effects, offering insights into the molecular basis of its sustained influence on plant growth and development. Looking ahead, future endeavours should aim to unravel the intricate web of interactions between auxin signalling and other plant hormones, such as cytokinins, to optimize auxin application strategies for enhanced propagation success. Additionally, the optimization of IBA concentrations across diverse plant species remains fertile ground for exploration, offering opportunities to tailor auxin treatments to specific physiological needs. By unravelling the physiological, metabolic and molecular intricacies of auxin signalling, we can unlock the full potential of IBA and refine auxin application strategies for sustainable and efficient plant propagation.

In exploring the intricate relationships between plant propagation traits, particularly in the context of rooting and bud break, recent scientific findings unveil a landscape rich in complexity and interconnectedness, akin to a delicate ecosystem governed by three distinct auxin types. Root length serves as a tangible indicator of efficient nutrient and water uptake, crucial for plant survival and establishment (50), while the emergence of roots signifies a stage of vigorous root system development, optimizing resource acquisition (51). Bud break dynamics mirror those of shoot development, indicating a coordinated effort driven by species-specific traits and hormonal cues (52). Positive correlations between rooting, bud break and root length underscore a feedback loop wherein successful rooting facilitates further elongation and shoot development, ensuring robust plant establishment (53). Exploring factors such as explant source, age and genetic variation elucidates additional layers of influence on propagation outcomes. Future avenues for research involve delving into gene expression patterns and hormone profiling to unravel the molecular mechanisms underpinning these intricate relationships, thereby refining propagation techniques for enhanced plant establishment across diverse species.

Conclusion

In conclusion, our study of *R. damascena* micropropagation reveals the distinct yet harmonious roles of auxins IAA, IBA and NAA in shaping plant growth. We observed rapid but fleeting rooting with NAA, steady growth with IAA and robust early rooting with IBA. These findings shed light on the intricate balance between rooting efficiency and bud break rates. During acclimatization, IBA-treated plants displayed superior growth, highlighting their potential in sustainable cultivation. The micropropagation of *R. damascena* through the application of auxins represents an innovative approach to improve root formation, acclimatization and potentially accelerate field transplantation to meet the rising demands for this beloved botanical treasure. Further studies are required to refine this technique for the large-scale production of *R. damascena*.

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Authors' contributions

S.E.M. and M.G. designed the study, conducted experiments, performed the statistical analysis and drafted the manuscript. M.E. supervised the acclimatization of plants and contributed to editing the document. H.T. managed agricultural practices and supported manuscript editing. M.L. oversaw the *in vitro* experiments and assisted with manuscript revision. F.E.E.K. assessed experimental attributes and provided technical support. M.M., D.H. and L.M.I.H. supervised the study, offered guidance and critically reviewed the manuscript for intellectual

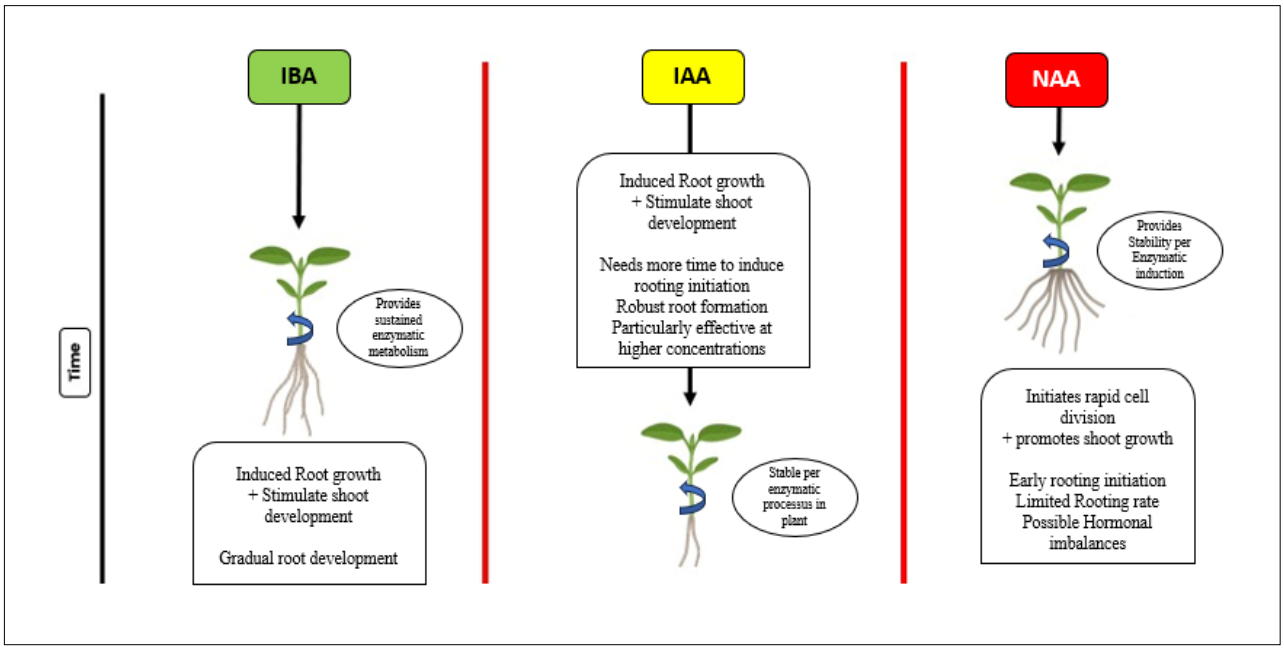

Fig.5. Comparative Effects of IBA, IAA and NAA mechanisms involved on Root Growth and Development in *R. damascena* Micropropagation

content. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

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

Ethical issues: None

Declaration of AI and AI-Assisted Technologies

While preparing this work, the authors utilized ChatGPT by OpenAI, to assist with the writing and editing process. This tool was employed to correct and to ameliorate the vocabulary and the language throughout the manuscript. Following the use of this tool, the authors thoroughly reviewed and edited the content as necessary and takes full responsibility for the final content of the publication.

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