https://doi.org/10.17221/192/2024-HORTSCI

Comparative evaluation of IBA and NAA effects on rhizogenesis and shoot morphogenesis in rose stem cuttings

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Citation: Soliman W.S., Saad-Eldeen K., Abbas A.M., Alshaharni M.O., Gahory A.A. (2026): Comparative evaluation of IBA and NAA effects on rhizogenesis and shoot morphogenesis in rose stem cuttings. Hort. Sci. (Prague), 53: 204–212.

Abstract: This study was conducted at the Floriculture Nursery, Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt, during the 2021/2022 and 2022/2023 growing seasons. The experiment aimed to evaluate the effects of two plant growth regulators (PGRs), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), on the root and shoot development of rose, a species known for its poor rooting ability. A split-plot arrangement within a randomised complete block design was used, with PGR type assigned to the main plots and concentrations (control, 500, 1 000, 1 500, and 2 000 ppm) allocated to the sub-plots. Both IBA and NAA significantly improved plant height, root length, and potassium content. The application of 500 ppm IBA and 1 000 ppm of NAA produced the greatest increases in rooting percentage, number of leaves and branches, shoot and root fresh and dry weights, and phosphorus content. Higher concentrations (1 500 and 2 000 ppm) reduced the time required for leaf sprouting and increased stem diameter as well as nitrogen content. Overall, concentrations between 500 and 1 500 ppm enhanced both shoot and root growth. However, soaking stem cuttings in a 2 000 ppm IBA solution for 30 minutes was the most effective for promoting root formation and improving overall plant growth.

Keywords: indole-3-butyric acid; naphthalene acetic acid; vegetative propagation; growth characters; rooting; shooting

Phytohormones are commonly classified into growth-promoting regulators such as auxins, gibberellins, cytokinins, brassinosteroids, and strigolactones, and stress-related hormones, including ethylene, salicylic acid, jasmonic acid, and abscisic acid (Gilroy, Breen 2022). Plant growth regulators (PGRs) are natural or synthetic substances that regulate plant growth through diverse physiological processes and are widely applied to enhance vegetative growth, propagation, and flowering (Pal 2019; Kumar et al. 2021).

Auxins are primarily synthesised in shoot and root apices and regulate cell elongation and differentiation. They accumulate in actively growing tissues such as coleoptile tips, buds, root tips, and young leaves. Synthetic auxins, particularly indole-

Supported by the Deanship of Research and Graduate Studies at King Khalid University, Large Group Project, Grant No. (RGP. 2/440/46).

https://doi.org/10.17221/192/2024-HORTSCI

3-butyric acid (IBA) and naphthaleneacetic acid (NAA), are widely used to improve root formation in stem cuttings and *in vitro* cultures (Pal 2019; Kumar et al. 2021).

IBA is a naturally occurring auxin that can be converted to indole-3-acetic acid (IAA) in many plants, highlighting its important physiological role. Due to its strong rooting effect, IBA is the most commonly used auxin in horticulture. It also regulates other auxin-mediated processes, including cell division, stem curvature, and leaf epinasty, even at sites distant from the point of application (El-Banna et al. 2023; Chen et al. 2025).

NAA is a synthetic auxin that may become toxic at high concentrations; however, at optimal doses, it effectively induces root formation and is often more potent than IAA, although less safe than IBA (Suman et al. 2017). NAA enhances IAA accumulation in cuttings and is more resistant to enzymatic degradation. It promotes cell elongation and stimulates cellular activity, resulting in larger plants and increased flowering yields (Palei et al. 2016). Previous studies have confirmed that IBA, NAA, and IAA, either individually or in combination, significantly improve root initiation in ornamental plant cuttings (Gowda et al. 2017).

The genus *Rosa* comprises approximately 200 species and more than 1 800 cultivars adapted to temperate and subtropical regions (Leus et al. 2018). They are of considerable economic importance in the fragrance, medicinal, ornamental, and cut-flower industries. The global rose market was valued at USD 525.94 million in 2023 and is expected to reach USD 1 092.77 million by 2032, with a compound annual growth rate of 8.7% during the period 2024 to 2032 (DataIntelo 2026). Rose hips contain bioactive compounds such as organic acids, flavonoids, carotenoids, and tannins, which have been valued since ancient times for their medicinal and cultural significance (Wang 2023). However, propagation through stem cuttings often results in low multiplication rates and poor rooting success.

Propagation by stem cuttings largely depends on the rooting ability of the plant material, which is strongly influenced by the type and concentration of auxins applied (Kaushik, Shukla 2020). Therefore, the present study aimed to evaluate the effects of IBA and NAA on rooting and shoot development in rose cuttings, a species known for its propagation difficulty.

MATERIAL AND METHODS

Experimental design and cutting propagation. This study was conducted during the 2021/2022 and 2022/2023 growing seasons at the Floriculture Nursery, Faculty of Agriculture and Natural Resources, Aswan University, Egypt (23°59'53.0''N 32°51'29.5''E). The experiment was arranged in a split-plot within a randomised complete block design with three replications, with each plot containing ten pots. The main-plot factor consisted of the PGRs IBA and NAA, while the sub-factor included five concentrations: control, 500, 1 000, 1 500, and 2 000 ppm. Each pot contained one cutting, resulting in a total of 30 pots (cuttings) per treatment and 300 pots per experiment.

Stem cuttings were collected on 25 February in both seasons from healthy five-year-old *Rosa polyantha* Thunb. mother plants. Cuttings were taken from the central portion of one-year-old branches and measured approximately 15 cm in length and 0.7 cm in diameter. The basal ends of the cuttings were dipped in IBA or NAA solutions for 30 min before planting in 18 cm diameter pots containing a mixture of peat moss, clay, and sand (1 : 1 : 1 v/v).

All pots were maintained under natural environmental conditions in a shaded area, receiving approximately 60% of full sunlight. The average temperature ranged between 35 °C and 37 °C, with ~35% relative humidity. Pots were irrigated daily to maintain substrate moisture close to field capacity, and the cuttings were regularly monitored to prevent desiccation.

Stock solutions of IBA and NAA were prepared by dissolving the compounds in a small volume of ethanol, followed by dilution with distilled water. The final concentrations (500, 1 000, 1 500, and 2 000 ppm) were freshly prepared before application. The pH of the PGR solutions ranged from 5.5 to 6.0. Control treatments received distilled water without PGRs.

Plant growth traits. After 12 months of cultivation, the cuttings were carefully uprooted, washed, and separated into shoots and roots. At the end of February in both seasons, the following parameters were recorded: cutting success percentage, days to leaf emergence, plant height, stem diameter, number of lateral branches and leaves per plant, root length, and shoot and root fresh and dry weights.

https://doi.org/10.17221/192/2024-HORTSCI

Photosynthetic pigments. Fresh leaf samples were collected from the middle canopy of each plant in each treatment to determine chlorophyll *a*, chlorophyll *b*, and carotenoid contents following the method of Metzner et al. (1965). Pigments were extracted using 80% aqueous acetone and measured with a SPECTROstar Nano spectrophotometer (BMG LABTECH GmbH, Germany) at wavelengths of 663, 644, and 452.5 nm.

Nitrogen, phosphorus, and potassium analysis. For chemical analysis, 0.2 g of dried shoot samples was wet digested with concentrated $H_2SO_4 : H_2O_2$ (1 : 1, v/v) in a heating digester (DK, Velp Scientific Srl, Italy). Nitrogen was determined using the semi-micro Kjeldahl method (Black et al. 1965). Phosphorus was measured using the vanadate-molybdate yellow colourimetric method at 470 nm, with potassium dihydrogen orthophosphate used as the standard. Potassium was determined by flame photometry using standard solutions (Chapman, Pratt 1961).

Statistical analysis. Data were analysed using analysis of variance (ANOVA) for a split-plot arrangement within a randomised complete block design. The factors included PGR type and concentration. The year was not included as a factor in the statistical model because the experimental conditions and treatment structure were identical across the two seasons (Snedecor, Cochran 1989). Before conducting ANOVA, the assumptions of normality and homogeneity of variances were verified using appropriate diagnostic tests. Significant differences among treatment means were determined using Tukey's honestly significant differences (HSD) test at $P \leq 0.05$. Pearson correlation coefficients were calculated to evaluate relationships among measured parameters. All statistical analyses were performed using JMP software (version 4; SAS Institute, Cary, NC, USA).

RESULTS

In this study, both IBA and NAA significantly increased cutting success and accelerated leaf emergence (Figure 1). The control treatment recorded a 15% success rate with an average sprouting time of 23 days. In contrast, IBA at 2 000 ppm achieved 76% success, with leaf emergence occurring after 16 days, approximately one week earlier than the control. NAA at 2 000 ppm reduced sprouting time to 19 days, four days earlier than the control.

Root development improved markedly with increasing PGR concentrations, as reflected by greater root length and biomass (Figure 2). Root length increased from ~4 cm in the control to more than 14 cm with 2 000 ppm NAA and about 16 cm with 2 000 ppm IBA. Root fresh and dry weights increased from about 6.0 g and 2.18 g in the control to 25.0 g and 9.3 g with IBA, and 19.0 g and 8.9 g with NAA at 2 000 ppm. These results indicate that IBA, particularly at 2 000 ppm, was more effective than NAA in promoting root growth.

Shoot traits, including plant height, stem diameter, number of branches, and number of leaves, were significantly influenced by both PGR type and concentration (Figure 2). At 2 000 ppm, IBA produced the longest shoots (~33.67 cm), the thickest stems (~3.86 mm), the highest number of leaves (47), and the greatest number of branches (4.67). In comparison, NAA at the same concentration produced lower corresponding values. Similarly, shoot fresh and dry weights were highest under the 2 000 ppm IBA treatment (13.3 and 10.7 g, respectively).

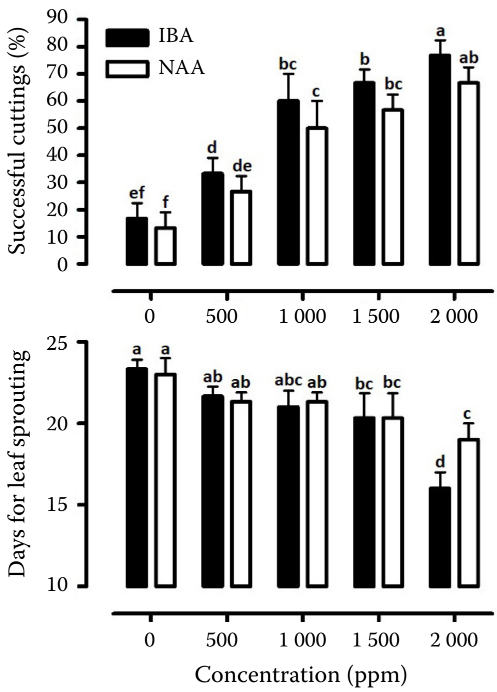

Figure 1. Effect of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) on successful cuttings percentage and leaf for leaf sprouting of rose

Different lowercase letters represent significant differences among treatments; values represented means of two seasons ± standard deviation (*n* = 6)

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Photosynthetic pigment contents were significantly affected by PGR concentration but not by PGR type or by the interaction between PGR type and concentration (Figure 3). Chlorophyll *a* and carotenoid contents increased with increasing PGR concentration, whereas chlorophyll *b* did not show significant changes. No significant differences were observed between IBA and NAA treatments, which may be attributed to environmental variability under open-field conditions that contributed to relatively high standard deviations.

Nutrient contents also varied depending on the treatments. Nitrogen content was significantly affected by both PGR type and concentration, phosphorus by all factors, and potassium by concentration and interaction effects. Overall, both IBA and NAA enhanced nutrient accumulation compared with the control, with IBA generally producing stronger growth responses (Figure 3).

Overall, both IBA and NAA enhanced rooting and vegetative growth compared with the control. Improvements in root development were accompanied by increased shoot growth, including greater plant height, stem diameter, leaf number, branch number, and shoot biomass. Across all evaluated parameters, IBA consistently produced stronger responses than NAA, particularly at 2 000 ppm.

DISCUSSION

The application of PGRs, particularly auxins, offers several practical advantages, including ease of application and environmental compatibility. However, their effectiveness is maximised when combined with appropriate cultural practices and ecological considerations to ensure sustainable production (Pal 2019; Kumar et al. 2021). In the present study, both IBA and NAA significantly improved cutting success and accelerated leaf emergence compared with the control. The highest concentrations advanced leaf emergence by up to one week and markedly increased the rooting

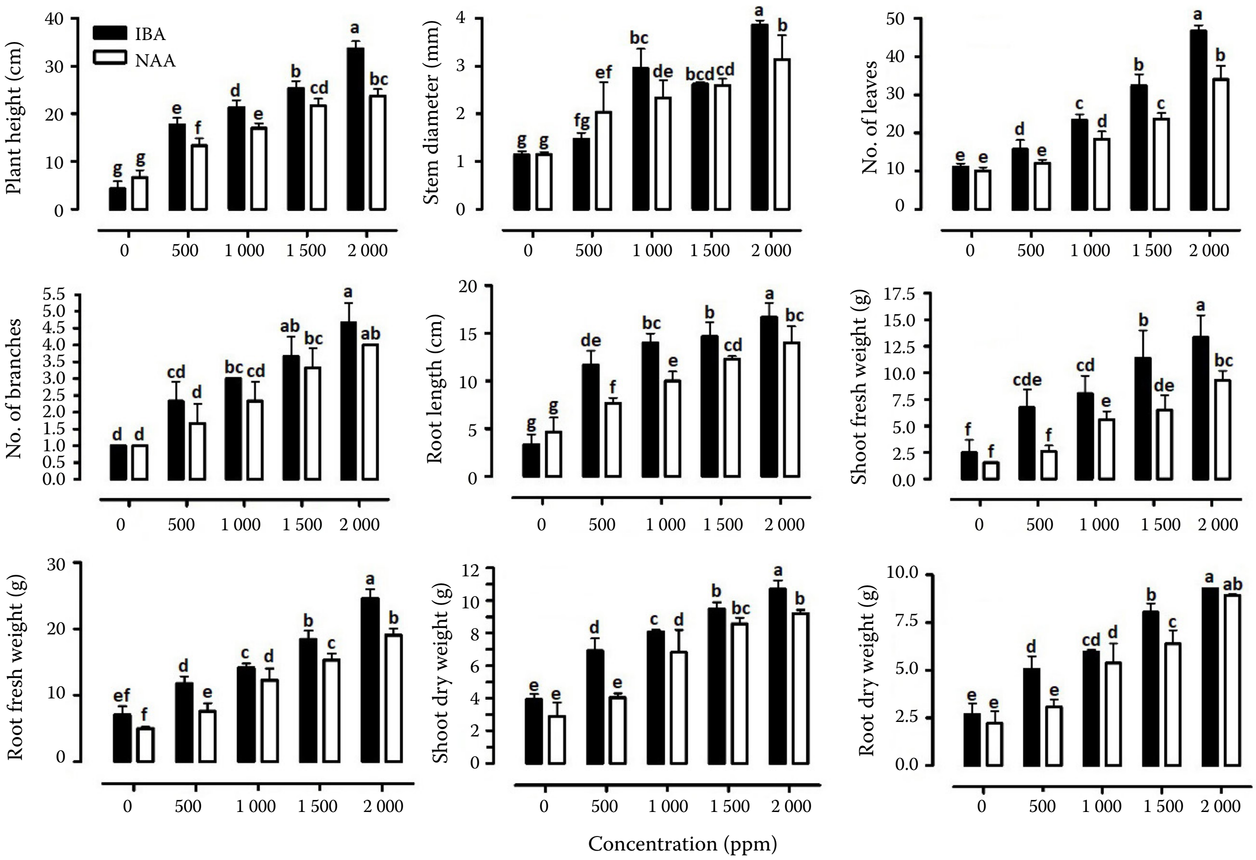

Figure 2. Effect of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) on plant height, stem diameter, number of leaves, number of branches, root length, and fresh and dry shoots and roots of rose

Different lowercase letters represent significant differences among treatments; values represented means of two seasons ± standard deviation (n = 6)

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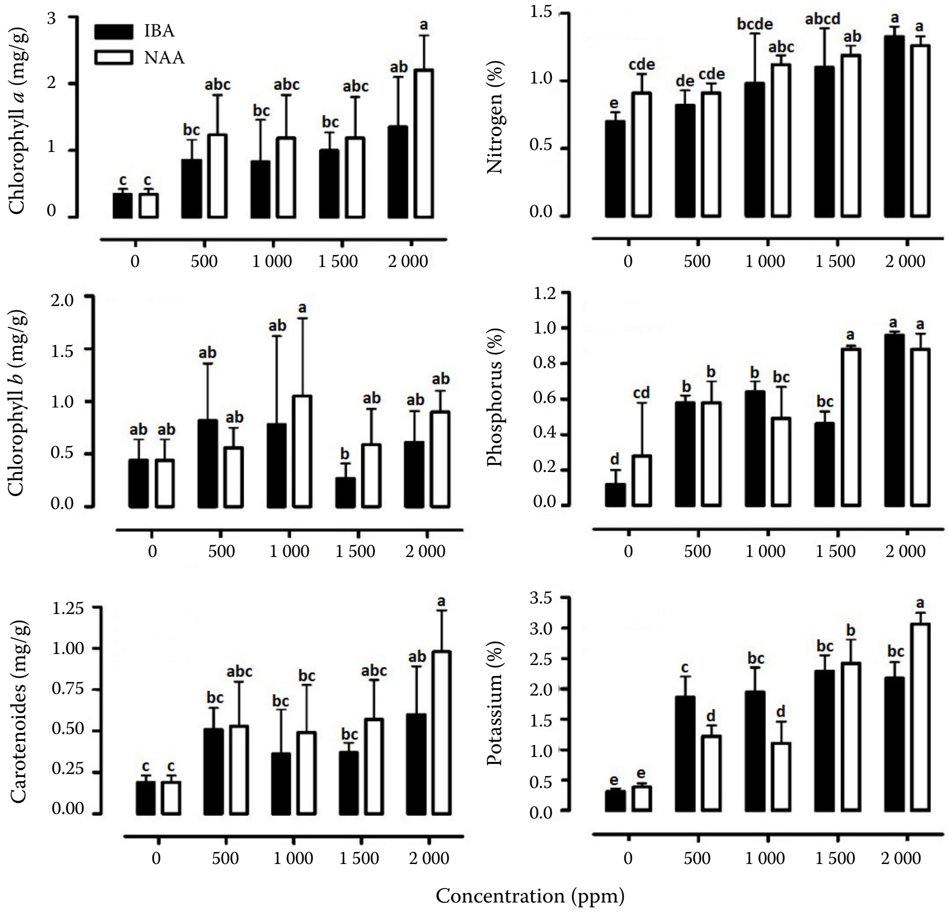

Figure 3. Effect of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) on photosynthetic pigments and macronutrients content of rose

Different lowercase letters represent significant differences among treatments; values represented means of two seasons ± standard deviation (*n* = 6)

success rate. These results are consistent with previous findings in rose (Abbas et al. 2015), *Jatropha curcas* (Kumari et al. 2023), and *Sanchezia speciosa* (Soliman et al. 2024).

Auxins play a central role in root development, and their physiological effects are concentration-dependent. Endogenous IAA levels have been reported to correlate inversely with plant growth rates (Nurzhanova et al. 2021). Auxin regulates gene expression through the ubiquitin–proteasome signalling pathway and interacts with other phytohormones, including gibberellins, thereby influencing cell wall formation and plant growth (Sofian et al. 2018). Among auxins, IBA is particularly effective in inducing root formation and is widely recommended for difficult-to-root species. Its positive effects on adventitious root formation have been confirmed in rose and other greenwood and hardwood cuttings (Nasri et al. 2015; Lei et al. 2018; Yang et al. 2019). In this study, IBA at 2 000 ppm outperformed NAA in promoting shoot growth, which agrees with recent reports (Chen et al. 2023; Kumari et al. 2023).

In stem cuttings, auxins penetrate through the cut surface and stimulate organ differentiation (Rahbin et al. 2012). Rooting capacity depends largely on the energy status of the cutting, which is influenced by photosynthetic efficiency, which is often reduced immediately after excision until new roots develop. Consequently, respiratory substrates play a crucial role during early root development (Soliman et al. 2024).

Significant positive correlations were observed between sprouting and rooting traits, consistent with a previous study (Chen et al. 2023). A shorter leaf emergence period was strongly associated with improved root and shoot growth. Increased root initiation facilitated nutrient uptake, photosyn-

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thesis, and carbohydrate accumulation, thereby resulting in more vigorous root systems and improved vegetative growth. Overall, IBA consistently showed superior performance compared with NAA (Figures 4 and 5).

IBA is widely recognised as a stable and highly effective auxin for inducing adventitious roots, largely due to its conversion into IAA within plant tissues (Lakehal, Bellini 2019). Transcriptomic studies have shown that IBA modulates genes involved in auxin homeostasis (Wei et al. 2019), including GH3 and IAA O-methyltransferase genes, which control free IAA levels through conjugation and methylation processes (Peat et al. 2012).

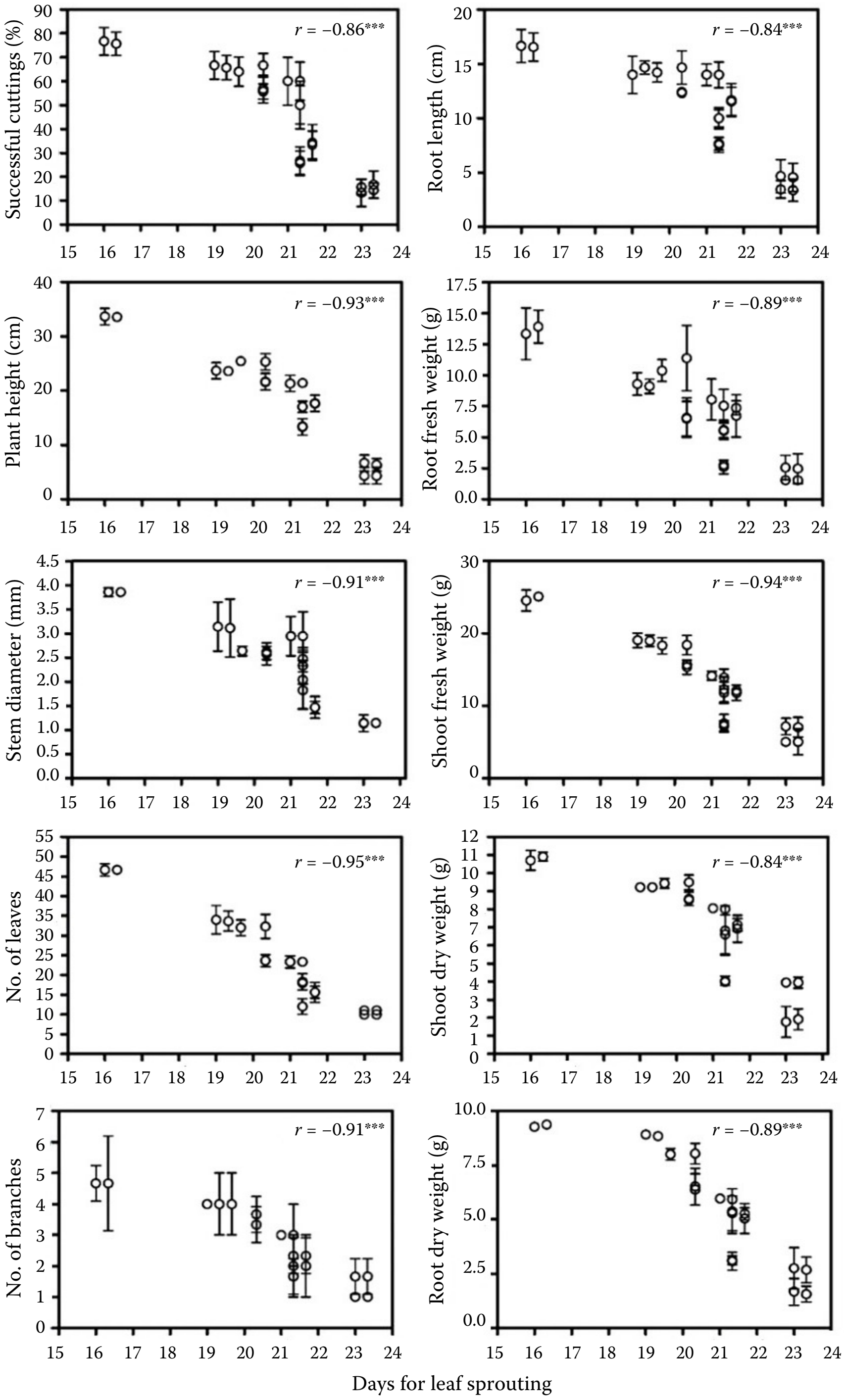

Figure 4. Correlation coefficient of days for leaf sprouting with shooting and rooting parameters in rose plants

***high significant correlation at P less than 0.001; values represent means of two seasons ± standard deviation (n = 6)

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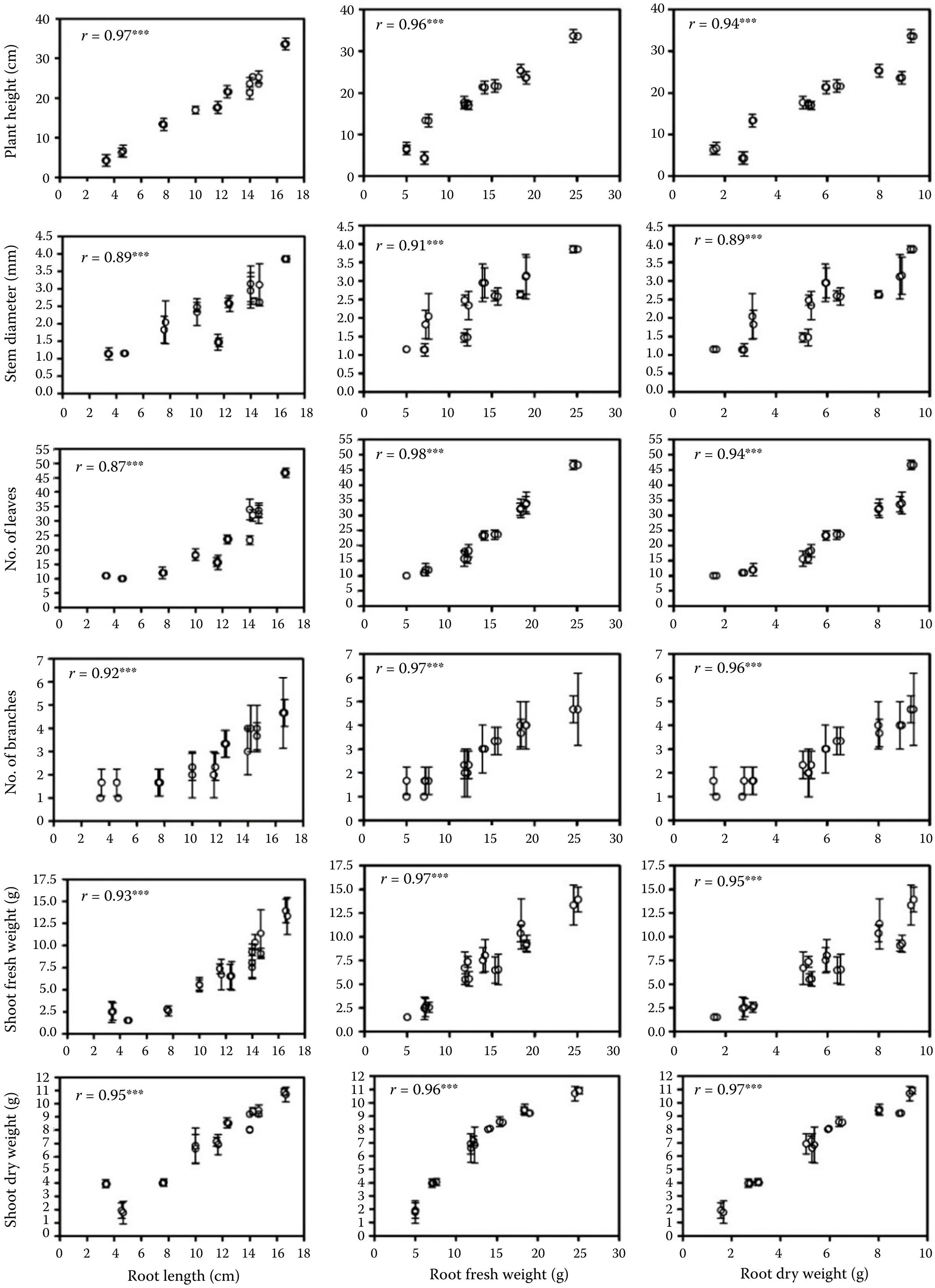

Figure 5. Correlation coefficient of rooting parameters and shooting parameters in rose plants

***high significant correlation at P less than 0.001; values represent means of two seasons ± standard deviation (n = 6)

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Similarly, exogenous NAA alters endogenous IAA balance, indicating feedback regulation. In tea plants, IBA and NAA have been shown to stimulate AR-related genes more effectively than IAA (Wei et al. 2019).

Adventitious root formation is regulated by IAA and its precursor IBA. Adventitious root formation begins with founder cell activation and cambial cell division near vascular tissues, which requires localised IAA accumulation (Guan et al. 2020). Polar auxin transport determines the positioning of root primordia, while IAA promotes cell division, expansion, and tissue differentiation (Wang et al. 2022; Tian et al. 2024). IBA contributes to both the initiation and elongation of root primordia (Tian et al. 2024). Directional auxin transport is mediated by PIN proteins, with PIN3 playing an important role in lateral auxin redistribution during growth responses (Ding et al. 2011).

Data availability: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Received: October 17, 2024
Accepted: March 18, 2026
Published online July 31, 2026