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Effect of auxins on root quality and anatomical integrity in tissue-cultured date palm (Phoenix dactylifera L.) cv. Mirhaj

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Abstract

Objective

Rooting represents a crucial phase in the micropropagation of date palm, and root quality is an important determinant of successful plantlet establishment. This study aimed to investigate the effects of two widely used auxins, indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), on rooting efficiency, root morphology, and anatomical structure in tissue-cultured date palm (Phoenix dactylifera L.) cv. Mirhaj. Special attention was given to identifying root malformations associated with different auxin types and concentrations, as well as understanding their anatomical characteristics.

Materials and methods

The experiment was conducted in 2024 using shoots derived from indirect somatic embryogenesis of cv. Mirhaj. Uniform shoots at the elongation stage were selected and cultured on full-strength semi-solid Murashige and Skoog (MS) medium supplemented with IBA or NAA at concentrations of 0.1, 0.25, and 0.5 mg L-1. Six treatments were arranged in a completely randomized design with fifteen replicates per treatment, where each shoot represented one replicate. Three shoots were cultured per 380 ml bottle containing 50 ml of medium. Cultures were maintained under controlled environmental conditions. After eight weeks, data were collected on rooting percentage, number of roots per shoot, root length, secondary root formation, and incidence of root malformations. For anatomical analysis, cross-sections of healthy and

malformed roots were prepared, fixed in 70% ethanol, stained with safranin and fast green, and examined under a light microscope.

Results

Auxin type and concentration significantly influenced rooting performance and root morphology. NAA at 0.25 and 0.5 mg L-1 produced the highest rooting percentage (90%) and increased the number of roots per shoot. In contrast, IBA at 0.1 mg L-1 resulted in the longest roots, indicating better elongation capacity. However, higher concentrations of NAA led to shorter roots and a marked increase in root malformations, reaching 50% at 0.5 mg L-1. No malformations were observed with low IBA concentrations. Anatomical analysis revealed severe structural abnormalities in malformed roots, including disrupted vascular tissues and irregular cellular organization.

Conclusion

The findings highlight a trade-off between rooting efficiency and root quality. While NAA improved rooting percentage, it negatively affected root structure. Low-concentration IBA, particularly 0.1 mg L^{-1}, promoted longer roots with no observed malformations under the conditions tested, suggesting its potential suitability for producing morphologically and anatomically sound roots.

Keywords: date palm, indole-3-butyric acid (IBA), Mirhaj cultivar, naphthalene acetic acid (NAA), tissue culture

Paper Type: Research Paper.

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Introduction

Date palm (Phoenix dactylifera L.) is one of the main crops in arid and semi-arid regions due to its economic and nutritional importance, as well as its role in supporting food security and stabilizing agricultural communities (Al-Khayri & Naik, 2017). The crop is well known for its ability to tolerate harsh environmental conditions, including high temperatures, water scarcity, and soil salinity, which makes it an essential agricultural species in many countries. In addition, date palm contributes significantly to the agricultural economy through the production of dates and related products. Its importance has increased further under climate change conditions, as it

represents a relatively sustainable production system in dry environments. Date palm micropropagation has been achieved through different in vitro pathways, including somatic embryogenesis, organogenesis, and the use of inflorescence explants, depending on genotype, explant source, and culture conditions (Bhaskaran & Smith, 1992; Abul-Soad, 2011; Ali et al., 2025). Although micropropagation offers several advantages, it still faces technical and economic limitations, particularly high production costs and relatively long production cycles (Maene & Debergh, 1985). Among the different stages, adventitious rooting and acclimatization are considered critical, since the survival of plantlets under field conditions depends largely on the efficiency of the root system. Poorly developed roots, either physiologically or anatomically, often result in low survival rates after transfer to soil (Nimavat & Parikh, 2024). Therefore, improving both rooting efficiency and root quality remains an important objective in date palm micropropagation. Root formation mainly depends on hormonal balance, especially auxins, which stimulate cell division in meristematic and pericycle-related tissues and lead to the initiation of root primordia (Hartmann et al., 2014). Among the commonly used synthetic auxins, indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) are widely applied to induce rooting in tissue culture systems. IBA is known to be gradually converted into the active form, indole-3-acetic acid (IAA), within plant tissues, which provides a more controlled hormonal effect and supports the development of well-formed roots (Epstein & Ludwig-Müller, 1993). In contrast, NAA is more stable, and this prolonged activity may lead to stronger cellular responses, especially at higher concentrations (Wang & Charles, 1991). However, increasing auxin concentration to enhance rooting does not always lead to better outcomes. In some cases, high concentrations may result in the formation of short, swollen, or anatomically abnormal roots, which reduces their ability to absorb water and nutrients. This suggests that rooting is not simply a linear response, but a process that is highly sensitive to hormonal balance (Pincelli-Souza et al., 2024). In addition, prolonged exposure to high levels of growth regulators may lead to genetic or epigenetic changes that appear as growth abnormalities (Duncan, 1997; Al-Khalifah & Askari, 2011). Maintaining hormonal balance is therefore important not only for rooting but also for tissue stability (Alzohairy et al., 2023). Based on this background, the present study focuses on evaluating the effect of auxin type and concentration on rooting efficiency and root quality in date palm, particularly in the local cultivar Mirhaj, which has not been extensively studied. Most previous studies have mainly focused on quantitative traits such as root number, while less attention has been given to anatomical characteristics, which are closely related to root function. Therefore, this study aimed to evaluate the effects of IBA and NAA on rooting, to examine root malformations anatomically, and to relate these changes to auxin type and concentration in order to identify suitable conditions for producing functional roots that support successful acclimatization.

Materials and methods

Study site and plant material: This study was conducted in 2024 at the laboratories of Jannat Al Nakheel Company, which specializes in the commercial production of date palm plantlets, in Baghdad Governorate, Republic of Iraq. Tissue-cultured shoots of date palm

(Phoenix dactylifera L.) cv. Mirhaj were used as plant material. These shoots were originally obtained through indirect somatic embryogenesis in 2019 and were maintained by continued multiplication to produce a sufficient number of plantlets for commercial production.

Culture medium and auxin treatments: Uniform shoots obtained during the elongation stage were cultured on full-strength semi-solid Murashige and Skoog (MS) medium (Murashige & Skoog, 1962). The medium was supplemented separately with two auxins, indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), at three concentrations for each auxin: 0.1, 0.25, and 0.5 mg L-1. Accordingly, six auxin treatments were evaluated: 0.1 mg L-1 IBA, 0.25 mg L-1 IBA, 0.5 mg L-1 IBA, 0.1 mg L-1 NAA, 0.25 mg L-1 NAA, and 0.5 mg L-1 NAA.

Experimental design and culture conditions: The experiment was arranged as a completely randomized design with six treatments and fifteen replicates per treatment, where each shoot was considered one replicate. Three shoots were cultured in each 380 ml culture bottle containing 50 ml of nutrient medium. The cultures were maintained under standard controlled tissue culture conditions of light, temperature, and humidity until data collection.

Data collection: After eight weeks of culture, the following rooting and morphological traits were recorded: rooting percentage (%), average number of roots per shoot, average root length (cm), percentage of secondary root formation (%), and percentage of root malformation (%). Malformed roots were identified visually based on abnormal swelling, irregular root shape, and apparent structural deformation.

Anatomical analysis: Following the rooting evaluation, root malformations were observed in some treatments as swollen structures that were likely to have reduced functional efficiency. To examine these abnormalities anatomically, healthy and malformed root samples were selected from rooted shoots of the Mirhaj cultivar (Figure 1). Samples were fixed in 70% ethyl alcohol, and transverse sections were prepared manually from the middle region of healthy roots and from the malformed region of abnormal roots. During sectioning, the root sample was held vertically between the thumb and forefinger and cut into thin sections using a sharp grafting blade until clear cross-sections were obtained. The sections were transferred to diluted sodium hypochlorite solution (1:0.5) for 5 minutes, stained with safranin prepared by dissolving 1 g of dye in 99 ml of 50% ethyl alcohol for 1–2 hours, and washed with 70% ethyl alcohol to remove excess dye. The sections were then counterstained with fast green for 5 seconds, followed by dehydration through 90%, 95%, and 99% ethyl alcohol. Sections were cleared using a mixture of 99% ethyl alcohol and xylene (1:1), followed by pure xylene. The prepared sections were mounted on glass slides and examined using a KRÜSS compound microscope. Photographs were taken using an AmScope MU1000 camera mounted on the microscope, following standard plant anatomical staining principles with minor modifications (Johansen, 1940; Ma et al., 1993).

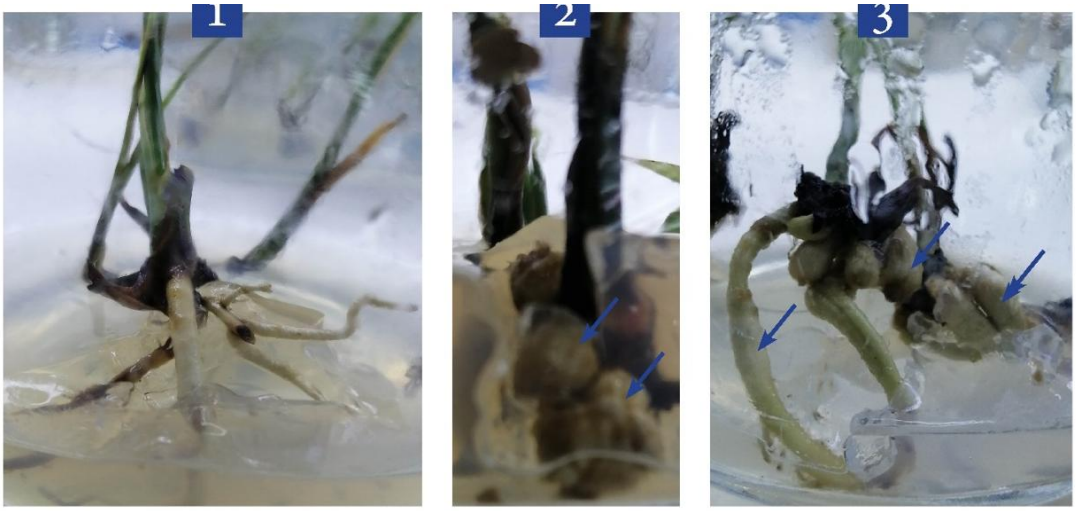

Figure 1. Rooted tissue-cultured shoots of date palm cv. Mirhaj. 1) healthy root formation; 2 and 3) malformed roots with visible swelling and abnormal root structure

Results

Effect of auxins on rooting of tissue-cultured shoots of date palm

Effect of auxins on rooting percentage: The results showed differences in rooting percentage among the different auxin treatments. NAA at concentrations of 0.25 and 0.5 mg L-1 produced the highest rooting percentage (90%), compared with IBA at 0.1 mg L-1, which recorded the lowest rooting percentage (60%) **(**Figure 2**)**.

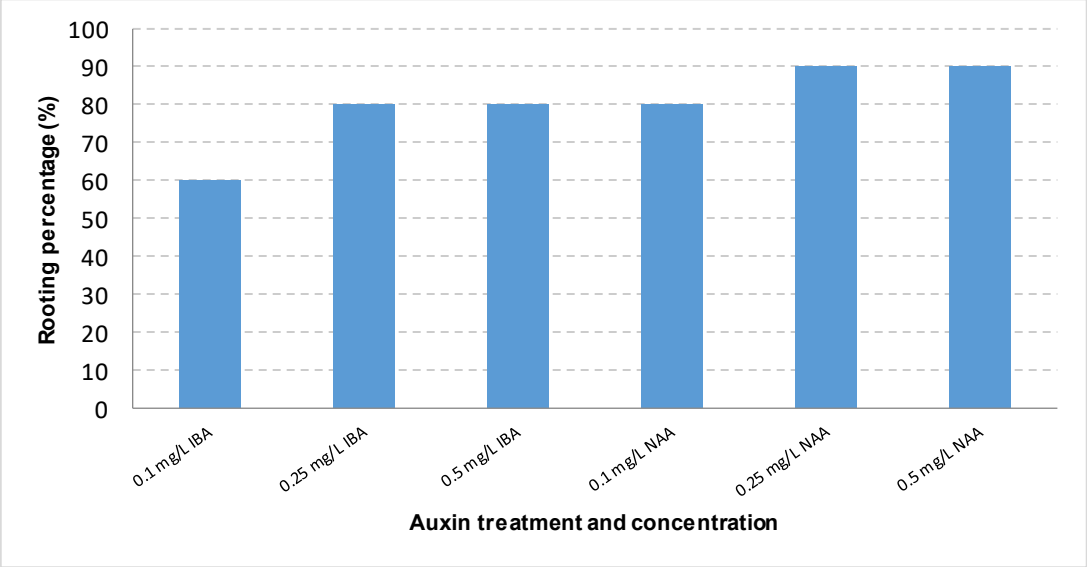

Figure 2. Effect of auxin type and concentration on the rooting percentage

Effect of auxins on average number of roots: The results shown in Figure 3 indicated that NAA treatments increased the average number of roots per shoot. NAA at 0.25 and 0.5 mg L-1

produced the highest values, reaching 4.51 and 4.30 roots per shoot, respectively, whereas IBA at 0.1 mg L-1 recorded the lowest average number of roots (2.45 roots per shoot).

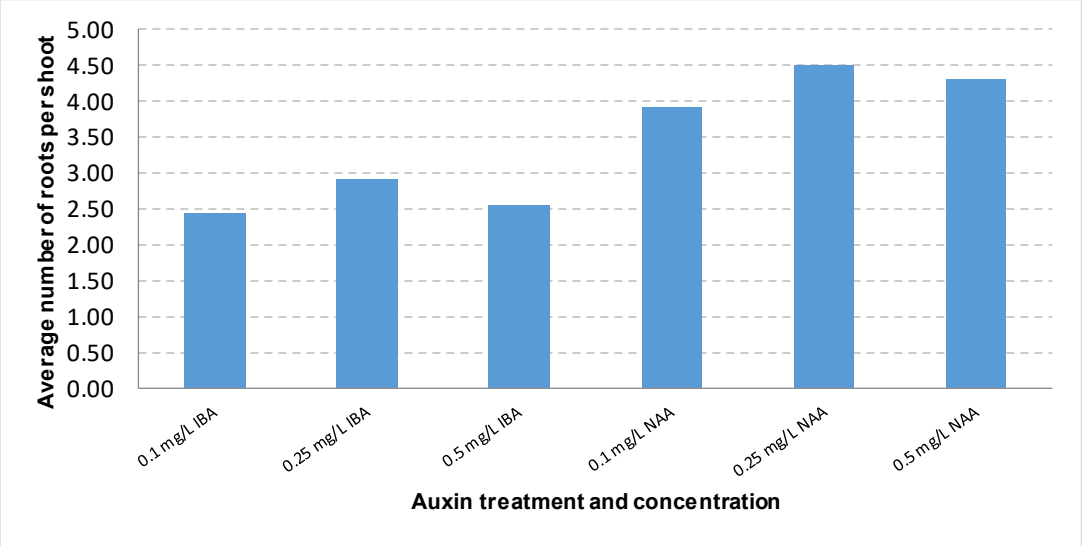

Figure 3. Effect of auxin type and concentration on the average number of roots per shoot

Effect of auxins on average root length: The results in Figure 4 demonstrated that IBA at 0.1 mg L-1 produced the highest average root length, reaching 4.45 cm, whereas NAA at 0.5 mg L-1 resulted in the lowest average root length (2.60 cm).

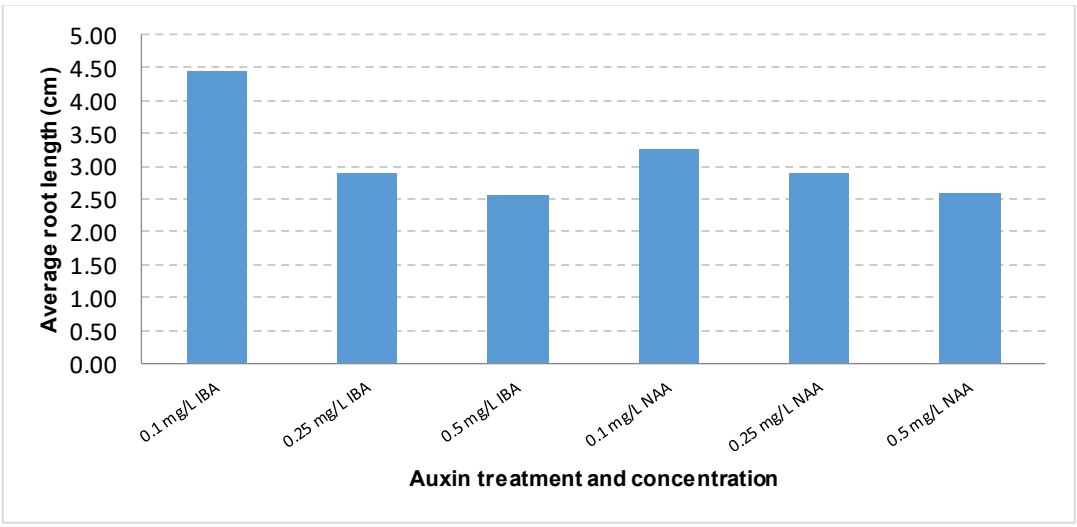

Figure 4. Effect of auxin type and concentration on average root length

Effect of auxins on percentage of secondary root formation: The results showed that NAA at 0.5 mg L-1 and IBA at 0.25 and 0.5 mg L-1 produced the highest percentage of secondary root formation (20%), whereas the remaining treatments recorded 10% (Figure 5).

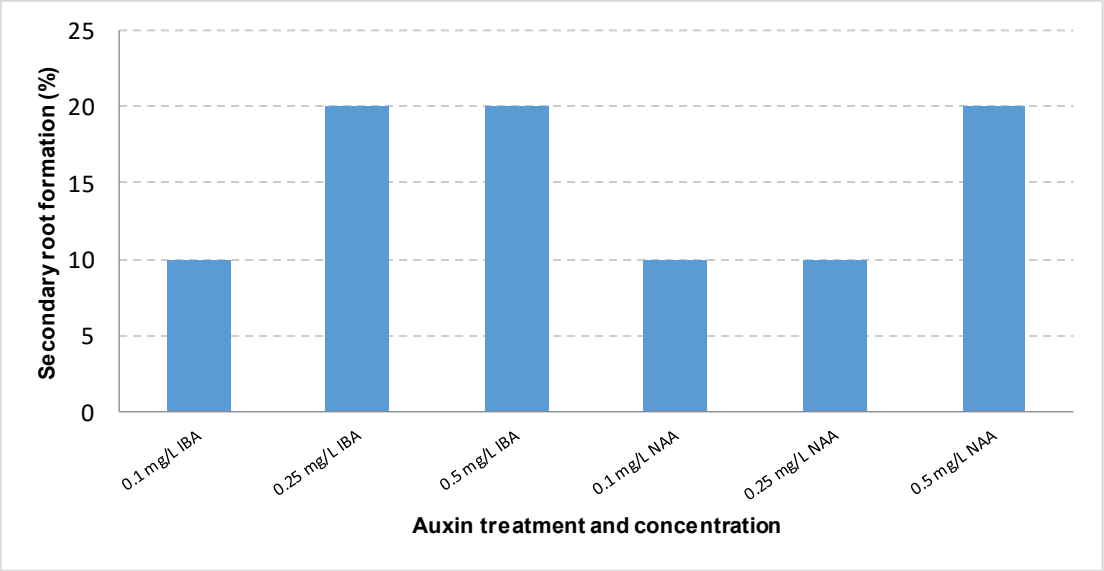

Figure 5. Effect of auxin type and concentration on percentage of secondary root formation

Effect of auxins on root malformation percentage: The results shown in Figure 6 revealed clear differences in root malformation percentage among the treatments. No root malformations were observed in IBA treatments at 0.1 and 0.25 mg L-1. In contrast, the percentage of root malformations increased with increasing NAA concentration, reaching 50% at 0.5 mg L-1.

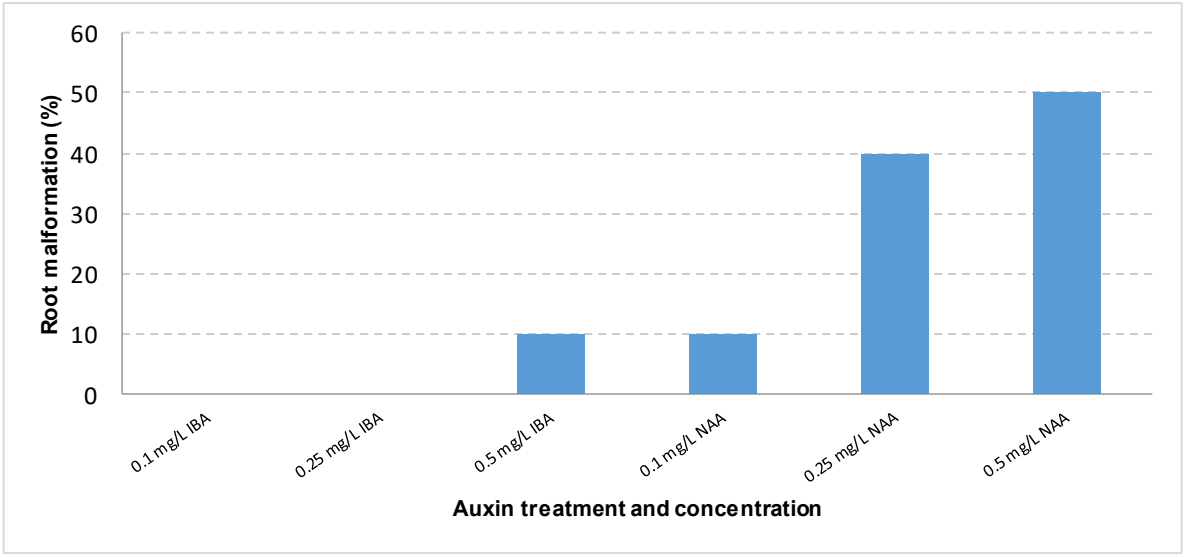

Figure 6. Effect of auxin type and concentration on root malformation percentage

Anatomical effects of auxins on date palm roots: The anatomical observations showed that treatment with NAA caused several abnormalities in root tissues formed during the rooting stage. Cross-sections of malformed roots showed general distortion in root structure, severe damage in the cortex region, rupture of parenchymatous tissues, disruption of the vascular cylinder, damage to xylem vessels, rupture of phloem tissues, deformation of sclerenchyma fibers,

and abnormal cell organization. The general distortion observed in the transverse sections may indicate disturbances in vascular tissue development and internal tissue organization. These abnormalities may be related to high auxin concentrations, which could induce swelling in root apical regions as a result of altered meristematic activity. Severe damage was also observed in the cortex region, accompanied by rupture of parenchymatous tissues, possibly due to inhibition or imbalance of meristematic tissues responsible for producing new cells. Consequently, new tissues were not properly formed, while older cells appeared weak and poorly connected. Mechanical separation resulting from unbalanced growth between the cortex and vascular cylinder may also have contributed to tissue rupture and internal peeling.

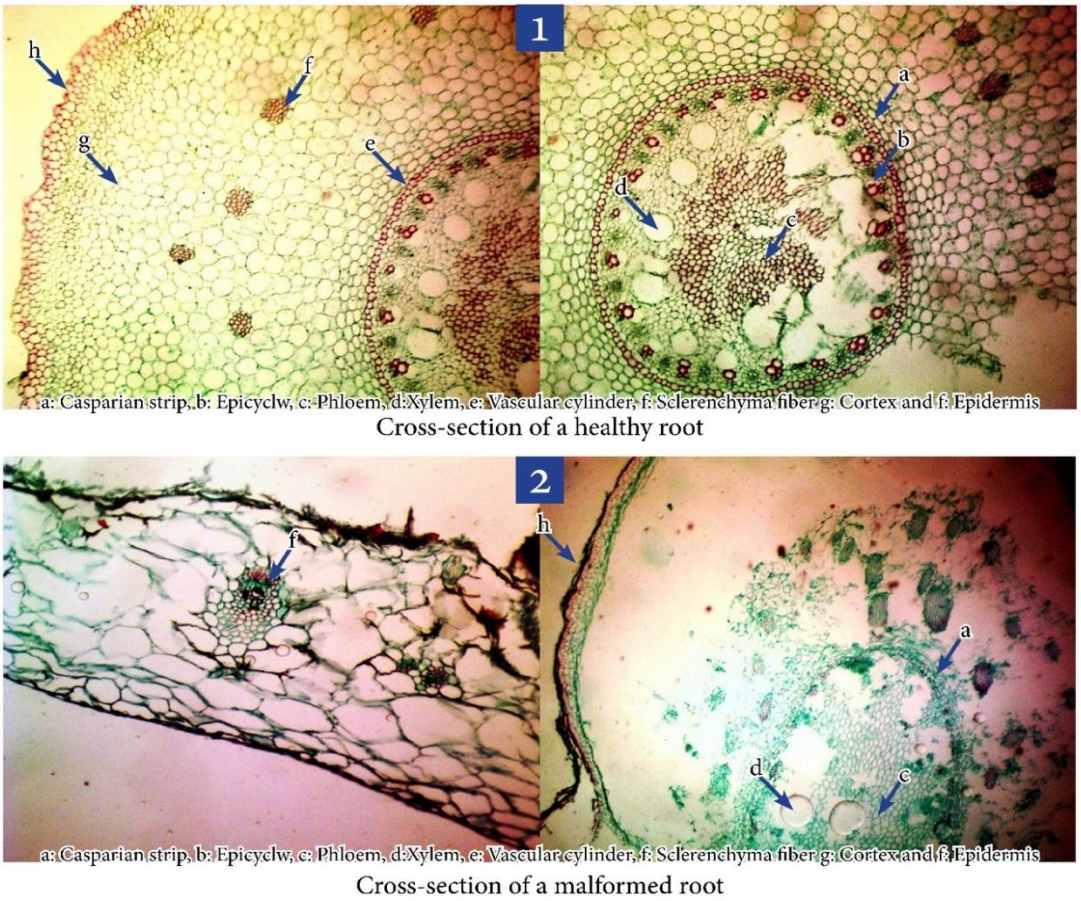

Figure 7. Anatomical cross-sections of date palm cv. Mirhaj roots showing. 1) healthy root with normal tissue organization; 2) malformed root showing cortical rupture, vascular cylinder disruption, and abnormal cellular arrangement

In addition, abnormalities were observed in the vascular bundles, including xylem and phloem, with reduced size and irregular arrangement within the vascular cylinder compared with the healthy root. Damage to sclerenchyma fibers and deformation of cells were also observed, leading to irregular thickening and the swollen appearance detected in malformed roots, particularly in the apical regions.

Discussion

The results of the present study showed that both auxin type and concentration had clear effects on rooting efficiency as well as on the morphological and anatomical characteristics of roots in tissue-cultured plantlets of date palm cv. Mirhaj. NAA treatments resulted in higher rooting percentage and a greater number of roots, which may be related to its relatively high chemical stability and prolonged activity within plant tissues, leading to increased stimulation of root primordia formation (Wang & Charles, 1991). In contrast, IBA was more effective in improving root quality and increasing root length. This effect may be attributed to its gradual conversion into the active form IAA, which provides a more balanced hormonal effect and supports normal root development (Epstein & Ludwig-Müller, 1993). These findings are consistent with the general principles of adventitious root induction, where auxin type, concentration, exposure period, and genotype interact to determine whether the response is beneficial or excessive (Hartmann et al., 2014; Pincelli-Souza et al., 2024). The current results also agree with previous work on date palm micropropagation showing that auxins are essential during rooting, but that the most suitable auxin regime is cultivar- and stage-dependent (Al-Khayri & Naik, 2017; Nimavat & Parikh, 2024). The increase in rooting percentage under NAA treatments is comparable with reports on date palm in which NAA improved root induction during the rooting stage. For example, Khierallah and Bader (2007) reported high rooting performance in date palm shoots cultured on medium containing NAA, whereas Tisserat (1984) also demonstrated that auxin-containing media could promote date palm root formation. However, the present study adds an important qualitative dimension by showing that higher rooting percentage does not necessarily indicate better root functionality when anatomical abnormalities are present. The superiority of low-concentration IBA for root elongation and anatomical integrity may be explained by its controlled metabolism and transport within plant tissues. IBA can act as a stable auxin reservoir that is gradually converted into IAA, thereby promoting root elongation without causing excessive localized auxin accumulation (Epstein & Ludwig-Müller, 1993). Similar positive effects of IBA on root induction and development have also been reported in date palm offshoot rooting, although responses may vary with cultivar, plant material, and application method (Afzal et al., 2011). The marked increase in root malformation percentage under higher NAA concentrations suggests that the prolonged activity of NAA may disturb normal cell division and tissue differentiation. Auxins stimulate cell division in meristematic regions and regulate vascular differentiation through xylem and phloem development. However, when auxin activity exceeds the optimum range, cell expansion and division may become unbalanced, resulting in swollen roots, abnormal cortex development, and vascular cylinder disruption. This interpretation is supported by the anatomical observations in the present study, where malformed roots showed ruptured cortical tissues, disorganized vascular tissues, damaged xylem vessels, ruptured phloem tissues, and deformation of sclerenchyma fibers. These anatomical findings are important because acclimatization success depends not only on root number, but also on the ability of roots to absorb water and nutrients after transfer to ex vitro conditions. Roots with disrupted vascular tissues or weakened cortex may be less efficient in water transport and mechanical support, which can increase plantlet mortality during acclimatization. Previous studies on tissue-cultured plants

emphasized that the transition from in vitro to ex vitro conditions is associated with physiological stress and that well-developed functional roots are critical for survival. The results also support the concept that date palm micropropagation should be evaluated using both quantitative and qualitative criteria. Many protocols focus primarily on rooting percentage, root number, or root length; however, anatomical integrity is equally important for determining whether the root system is functional. This is particularly relevant in date palm, where commercial propagation depends on producing uniform, vigorous, and acclimatization-ready plantlets (Jain et al., 2011; Abahmane, 2011; Fki et al., 2011). Therefore, selecting a rooting treatment based only on the highest root number may lead to poor ex vitro performance if the roots are anatomically abnormal. Overall, the present study indicates that NAA improves rooting efficiency in quantitative terms, whereas low concentrations of IBA provide better root quality and anatomical integrity. This distinction explains the apparent trade-off observed between rooting percentage and root functionality. For cv. Mirhaj, IBA at 0.1 mg L-1 appears more suitable for producing longer and structurally normal roots, while higher concentrations of NAA should be avoided when the objective is to produce plantlets with greater potential for successful acclimatization. A key contribution of the present study is the integration of quantitative rooting traits with morphological and anatomical assessment of root quality in tissue-cultured date palm cv. Mirhaj. By demonstrating that a higher rooting percentage does not necessarily correspond to superior root quality, the study provides a more comprehensive basis for selecting auxin treatments and highlights the importance of anatomical integrity when optimizing rooting protocols for date palm micropropagation.

Conclusions: NAA improved rooting efficiency by increasing rooting percentage and root number; however, high concentrations were associated with root malformations characterized by swelling and may have reduced functional efficiency. IBA, particularly at 0.1 mg L-1, showed higher efficiency in promoting longer roots with normal anatomical structure, making it more suitable for producing functionally efficient roots in tissue-cultured date palm cv. Mirhaj. Root malformations were characterized by clear anatomical abnormalities, including rupture of the cortex and vascular cylinder, as well as damage to xylem vessels, phloem tissues, and sclerenchyma fibers. Based on these findings, the use of IBA at a low concentration (0.1 mg L-1) is recommended during the rooting stage because of its role in producing long, anatomically normal, and functionally efficient roots. High concentrations of NAA should be avoided during rooting because they increased root malformation and reduced root quality. Malformed roots should be removed before acclimatization because of their low functional efficiency and limited ability to support plant growth after transfer to soil. Further studies are recommended to investigate the relationship between root malformations and physiological functions such as absorption efficiency, water transport, and plant survival after transfer to field conditions. Additional research on other local date palm cultivars is also recommended to develop more efficient propagation protocols.

Author contributions

All authors contributed equally to the conception, design, implementation, data collection, analysis, and writing of the manuscript. All authors read and approved the final version of the manuscript.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Ethical approval

Not applicable. This study was conducted on plant material and did not involve human participants or animals.

Conflict of interest

The authors declare that they have no conflict of interest.

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مجله بیوتکنولوژی کشاورزی
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تأثیر اکسین‌ها بر کیفیت ریشه و یکپارچگی آناتومیکی در نخل خرمای کشت‌شده در شرایط کشت بافتی رقم میرهاج (*Phoenix dactylifera* L.)

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چکیده

هدف: ریشه‌زایی مرحله‌ای حیاتی در ریزازدیادی نخل خرما محسوب می‌شود و کیفیت ریشه یکی از عوامل تعیین‌کننده مهم در استقرار موفق گیاهچه است. این مطالعه با هدف بررسی اثرات دو اکسین پرکاربرد، ایندول-۳-بوتیریک اسید (IBA) و نفتالین استیک اسید(NAA) ، بر کارایی ریشه‌زایی، مورفولوژی ریشه و ساختار آناتومیکی در نخل خرمای (*Phoenix dactylifera* L.)رقم میرهاج کشت‌شده در شرایط کشت بافتی انجام شد. توجه ویژه‌ای به شناسایی ناهنجاری‌های ریشه مرتبط با انواع و غلظت‌های مختلف اکسین‌ها و همچنین درک ویژگی‌های آناتومیکی آن‌ها معطوف شد.

مواد و روش‌ها: این آزمایش در سال ۲۰۲۴ با استفاده از شاخساره‌های حاصل از جنین‌زایی سوماتیکی غیرمستقیم رقم میرهاج انجام شد. شاخساره‌های یکنواخت در مرحله طویل‌شدن انتخاب و در محیط نیمه‌جامد Murashige and Skoog (MS) با غلظت کامل، حاوی IBA یا NAA با غلظت‌های ۰/۱، ۰/۲۵، و ۰/۵۰ میلی‌گرم در لیتر کشت شدند. شش تیمار در قالب طرح

کاملاً تصادفی با ۱۵ تکرار برای هر تیمار تنظیم شدند که در آن هر شاخساره به‌عنوان یک تکرار در نظر گرفته شد. سه شاخساره در هر بطری ۳۸۰ میلی‌لیتری حاوی ۵۰ میلی‌لیتر محیط کشت قرار داده شدند. کشت‌ها تحت شرایط محیطی کنترل‌شده نگهداری شدند. پس از هشت هفته، داده‌های مربوط به درصد ریشه‌زایی، تعداد ریشه در هر شاخساره، طول ریشه، تشکیل ریشه‌های ثانویه و میزان بروز ناهنجاری‌های ریشه جمع‌آوری شد. برای تجزیه‌وتحلیل آناتومیکی، مقاطع عرضی از ریشه‌های سالم و دارای ناهنجاری تهیه، در اتانول ۷۰ درصد تثبیت، با سافرانین و فست گرین رنگ‌آمیزی و با استفاده از میکروسکوپ نوری بررسی شدند.

نتایج: نوع و غلظت اکسین تأثیر معنی‌داری بر عملکرد ریشه‌زایی و مورفولوژی ریشه داشت. NAA با غلظت‌های ۰/۲۵ و ۰/۵۰ میلی‌گرم در لیتر بیشترین درصد ریشه‌زایی (۹۰٪) را ایجاد کرد و تعداد ریشه‌ها در هر شاخساره را افزایش داد. در مقابل، IBA با غلظت ۰/۱ میلی‌گرم در لیتر منجر به تولید بلندترین ریشه‌ها شد که نشان‌دهنده ظرفیت بهتر برای طویل‌شدن ریشه بود. بااین‌حال، غلظت‌های بالاترNAA موجب کوتاه‌تر شدن ریشه‌ها و افزایش قابل توجه ناهنجاری‌های ریشه شد؛ به‌طوری‌که میزان ناهنجاری‌ها در غلظت ۰/۵۰ میلی‌گرم در لیتر به ۵۰٪ رسید. در غلظت‌های پایین IBA هیچ‌گونه ناهنجاری مشاهده نشد. تجزیه‌وتحلیل آناتومیکی، ناهنجاری‌های ساختاری شدیدی را در ریشه‌های دارای ناهنجاری نشان داد که شامل اختلال در بافت‌های آوندی و سازمان‌یافتگی نامنظم سلولی بود.

نتیجه‌گیری: یافته‌های این پژوهش وجود یک توازن میان کارایی ریشه‌زایی و کیفیت ریشه را نشان می‌دهد. اگرچه NAA درصد ریشه‌زایی را بهبود بخشید، اما بر ساختار ریشه اثر منفی داشت. IBA با غلظت پایین، به‌ویژه ۰/۱ میلی‌گرم در لیتر، تحت شرایط مورد بررسی موجب تولید ریشه‌های بلندتر و بدون ناهنجاری مشاهده‌شده شد؛ بنابراین، این غلظت می‌تواند برای تولید ریشه‌هایی با ساختار مورفولوژیکی و آناتومیکی سالم مناسب باشد.

کلمات کلیدی: ایندول-۳-بوتیریک اسید (IBA)، رقم میرهاج، کشت بافت، نخل خرما، نفتالین استیک اسید (NAA)

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