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Nitric oxide enhances rooting of Pendolino olive cuttings by upregulating CAT, POD, and PPO activities while suppressing IAA oxidase, phenols and total sugars

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Abstract

Vegetative propagation is crucial for maintaining genetic integrity in the production of commercial olive planting material. However, poor rooting makes it difficult to grow cuttings from specific varieties. Nitric oxide (NO), a signalling molecule that has been shown to enhance root initiation in various plant species, may help overcome this barrier. This study evaluated the effect of nitric oxide (NO) at concentrations of 0, 50, 100, 500, 1000, 2000, 4000, and 8000 µM on the rooting of Pendolino olive cuttings. NO at 100 µM produced the highest rooting percentage (52.24%), number of roots (10.8), and root length, alongside significant increases in catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO) activities, and a marked reduction in indole-3-acetic acid oxidase (IAAox) activity. In comparison to the control (0 µM NO + 4000 ppm IBA), 100 µM NO elevated CAT, POD, and PPO activities by 52%, 85.22%, and 88.51%, respectively, while reducing IAAox by 44%. At this concentration, NO also decreased total phenols by 9.14%. The dose-dependent pattern of the effect of NO concentration on roots could be distinguished. Rooting was significantly inhibited by the highest concentration of NO (8000 µM). These results indicate that neither suboptimal nor supraoptimal NO concentration can promote effective adventitious rooting, highlighting the importance of a precise concentration range for improved rooting in challenging olive cultivars.

Key words: *Olea europaea*, phenol, sugars, CAT, POD, IAAox, PPO, indole

Introduction

Olive (*Olea europaea* L.) trees are regarded as one of the oldest fruit trees cultivated in the Pharaohs' era. The Mediterranean region is regarded as the main olive-producing region, but olives are cultivated in many countries worldwide. In 2023/2024, the worldwide production of table olives reached 2,564,000 tons, with Egypt contributing around 23.4% (IOC, 2025). Egypt ranks as the third-largest exporting country outside the European Union (IOC, 2025), with a cultivated area of 114,102 hectares and a total production of 1,034,309 tonnes (FAO, 2023).

Leafy or hardwood cuttings are the most common propagation methods for asexual olive propagation (Mohammed, 2021; Eid *et al.*, 2018; Rashedy *et al.*, 2021; Rashedy, 2022). The rising demand for olive consumption and planting, driven by an increasing population, economic importance, human health benefits, and the organoleptic properties of olive oil, has led to an increase in the demand for olive transplants (Gouvinhas *et al.*, 2017). Olive cultivars can be classified between easy to root such as Coratina (Rashedy, 2022) and hard to root such as Kalamata (Denaxa *et al.*, 2021) and between the previous classification, such as Picual, Manzanillo (Rashedy *et al.*, 2021) and Pendolino (Fabbri *et al.*, 2004). IBA application is a must in all olive cultivars. Moreover, the economically important Pendolino olive oil cultivar, of Italian origin, showed intermediate or even poor rooting capacities (Fabbri *et al.*, 2004).

Synthetic IBA is regarded as the most effective growth regulator for stimulating the rooting of olive cuttings (Al-Allaf, 2009; Hamdani and Mohammed, 2017; Rashedy *et al.*, 2021, Rashedy, 2022). Nitric oxide (NO) is a diatomic free radical gas that can be synthesized via either an enzymatic or non-enzymatic pathway (Skiba *et al.*, 1993; Rockel *et al.*, 2002). It has various critical roles in regulating many physiological processes such as seed germination (Beligni and Lamattina, 2000; Wang *et al.*, 2015), stomatal closure (Neill *et al.*, 2008; Shi *et al.*, 2014), pollen tube growth (Wang *et al.*, 2009), senescence (Liao *et al.*, 2013), and root development (Liao *et al.*, 2011; Corpas and Juan, 2015). NO at 100 µM stimulates rice root formation by cross-talk with ethylene and modulation of Auxin homeostasis (Kushwaha *et al.*, 2019). Additionally, NO at 1000 mg/L together with 500 mg/L K-IBA for 3 h stimulates root growth of *Eucalyptus grandis* (Abu-Abied *et al.*, 2012), chrysanthemum at 100 µM (Liao *et al.*, 2010), and *Panax ginseng* at 100 µM (Tewari *et al.*, 2008). Chen *et al.* (2024) reported a significant positive relationship between the rooting of tea cuttings and enzyme activity (CAT, PPO, POD). Additionally, they found an increase in OPP activity and a decrease in IAAox activity during root formation.

Moreover, NO is regarded as a downstream messenger for auxin, which induces adventitious root formation (Liao *et al.*, 2009; Li *et al.*, 2020; Dave *et al.*, 2012). Exogenous Auxin at the basis of cuttings, increases their NO production and accumulation in cells

that develop into root primordia (Xu *et al.*, 2017). However, NO application in tea cuttings was able to induce adventitious root formation (Kang *et al.*, 2018). The effects of NO depend on its concentration, genotype sensitivity (Sarropoulou *et al.*, 2015) and the time of application (Liao *et al.*, 2010). There is limited research on the effect of NO on the rooting of olive cuttings. Therefore, this study aimed to investigate the impact of different NO concentrations on the rooting of Pendolino olive cuttings.

Materials and methods

This experiment was carried out in the nursery of the faculty of Agriculture (Pomology Department), Cairo University, Egypt (30°01'04"N31°12'30"E).

Plant materials: Three 45-year-old stock trees of Pendolino olive cultivars were selected for preparing the hardwood cuttings. In December, cuttings of Pendolino olive cv were prepared, each with a 15 cm length and 1 cm width, and with 4 nodes. All leaves removed except for the top 4 leaves.

Treatments: The prepared cuttings were divided into nine groups, with 75 cuttings for each treatment (25 cuttings per replicate). Sodium nitroprusside was used as a source for NO, which was dissolved in distilled water to prepare solutions of different concentrations. After preparation, each group of cuttings (75 cuttings) was individually soaked for 30 minutes in the following freshly prepared NO concentrations. Sodium nitroprusside (1188 mg) was dissolved in 500 mL of distilled water to prepare a solution of 8000 µM of NO, and then other concentrations were prepared (0, 50, 100, 1000, 2000, 4000 µM). Then, at planting time, all cuttings were soaked for 20 seconds in IBA at 4000 ppm (Rashedy, 2022). All cuttings were immediately planted in a plastic perforated box (50x70 cm) containing a 4:1 (v/v) mixture of sand and peat moss under a closed polyethylene tunnel system in a saran-shaded (40% shade net) greenhouse as an alternative to the fog irrigation system (Hussein *et al.*, 2020; Rashedy *et al.,* 2021).

Observations: In April (120 days post-planting during the winter season), the cuttings were removed and the following parameters were determined:

Rooting behaviour: It included rooting percentage ((Number of rooted cuttings/total number of cuttings) × 100), root length (cm), and number of roots per cutting.

Biochemical analysis: After one week of planting, samples of cuttings for each treatment were collected and stored in a refrigerator at -30 °C. At the time of analysis, samples were collected from the bark at the bases of the cuttings as three replicates for the following analysis.

Total indole content: Plant samples were mixed with 4 mL of *p*-dimethylaminobenzaldehyde (PAB) mixture, which was prepared by dissolving 1 g of PAB in 50 mL of 95% ethanol and 50 mL of HCl. This mixture was incubated for 90 minutes at 30°C, and then the total indoles were determined using a spectrophotometer at 530 nm, expressed as mg g^{-1} FW (Fresh weight) of acetic acid (Larsen *et al.*, 1962).

Total phenols: A half gram of bark samples was extracted using methanol (80%). One milliliter of the filtered extract was placed in a tube for testing. Then, 1 mL of Folin was added, followed by 5 mL of Na_2CO_3 (20%), and finally, 3 mL of distilled water was added. After that, this mixture was kept in the dark for 60 minutes, and then total phenols were measured at 795 nm and expressed as equivalents of gallic acid in mg g^{-1} FW (Sharma *et al.*, 2019).

Total sugar content: It was determined in a plant sample (0.5 g) that was extracted using ethanol (70%). After that, 1 mL of phenol (5%) and then 4 mL of H_2SO_4 (98%) were added to the previous ethanolic extract in a test tube, and the reaction was allowed to proceed for 60 minutes before being stopped. Finally, total sugars were determined at 490 nm using a spectrophotometer and expressed as mg g^{-1} FW (Dubois *et al.*, 1956).

Enzyme activity

Enzyme extract was prepared by homogenizing in cold phosphate buffer (pH 6.8-7.5), adding EDTA and DTT/β-mercaptoethanol, and then centrifuging (10,000–15,000 × g for 15-30 minutes at 4°C). The supernatant was used as the enzyme extract.

Catalase (CAT): The decrease in absorbance was measured using a UV-Vis spectrophotometer over 1-3 minutes at 240 nm after mixing the enzyme extract with phosphate buffer (pH 7.0) and H_2O_2 (Aebi, 1984). One unit of CAT activity = decrease of 0.01 absorbance units per minute at 240 nm.

Peroxidase (POD): It was determined via mixing enzyme extract with phosphate buffer, guaiacol (substrate), and H_2O_2then the increase in absorbance (470 nm) was recorded (Chance and Maehly, 1955). One unit of POD activity = increase in absorbance of 0.01 per minute at 470 nm.

Polyphenol oxidase (PPO): The enzyme activity was determined using a phosphate buffer containing catechol, and the increase in absorbance (at 420 nm) over time was recorded. One unit of PPO activity is defined as the amount of enzyme that causes an increase in absorbance of 0.01 per minute at 420 nm under the assay conditions (Mayer *et al.*, 1965).

Indole-3-acetic acid oxidase (IAAox): The determination was made by incubating the enzyme extract with IAA and manganese sulfate in phosphate buffer. Stop reaction after a fixed time (30 min) with an acid reagent ($FeCl_3$-$HClO_3$) and IAAox was measured at 530 nm using a spectrophotometer (Mahadevan and Sridhar, 1982).

Statistical analysis: The treatments were arranged in a completely randomized block design with one factor (NO) and 8 levels (NO concentrations). Each treatment included 3 replicates. The least significant differences between treatments were analysed by ANOVA using the MSTAT-C package (Freed *et al.*, 1990). The least significant differences between treatments were calculated at a 0.05 level (Snedecor and Cochran, 1989).

Results and discussion

Nitric oxide (NO) concentration significantly influenced both rooting (%), root number and root length in olive cuttings (Fig. 1) .

Rooting (%): The effect of increasing concentrations of nitric oxide (NO) on rooting percentage of Pendolino olive cutting was evaluated (Fig. 1a). Rooting was lowest in the control and increased substantially with 50 µM NO (14.02%). Maximum rooting was observed at 100 µM, with a percentage of 52.24%. As the NO concentration increased beyond 100 µM, the rooting

percentage declined, with 500 µM resulting in 30%. Further increases in NO led to progressively lower rooting percentages: 2000 µM and 4000 µM both yielded 21.02%, and the highest concentration (8000 µM) produced the lowest rooting percentage (5.03%).

Root number: The influence of various concentrations of nitric oxide (NO) on root number in olive cuttings also exhibited a trend similar to that of rooting percentage (Fig. 1b). The highest values were recorded at 100 µM (10.8) and 500 µM (8.68), which were statistically similar. The next group included 1000 µM (8.67), followed by 50 µM (8.06) and 2000 µM (7. 1). The lowest root numbers were recorded 8000 µM (e), and the control 0 µM (1.33).

Root length: Root length (Fig. 1c) was affected by treating with NO in all concentrations. For root length, the longest roots were produced at 500 µM (10.59 cm) and 100 µM (8.81 cm), which did not differ significantly. These were followed by 1000 µM, while 2000 µM and 4000 µM had declining effect on root length. The shortest roots occurred at 8000 (5.85 cm) µM and the control (1.33 cm).

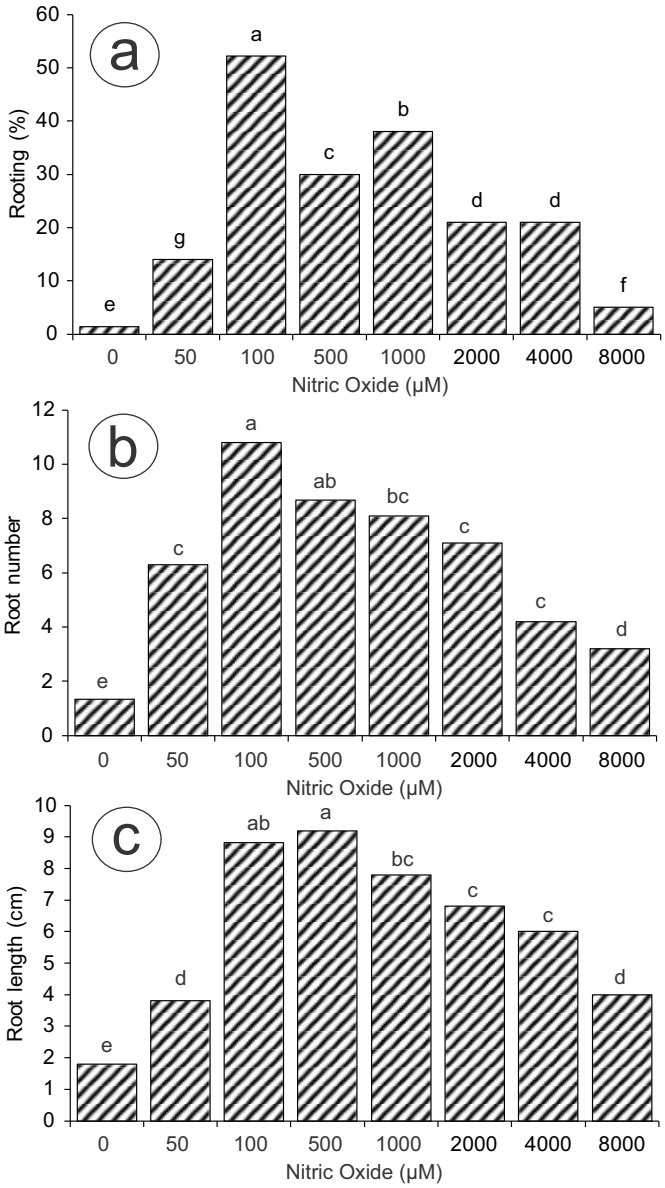

Fig. 1. Effect of different NO concentrations (0, 50, 100, 500, 1000, 2000, 4000, 8000 µM) on rooting percent (a), root number (b), and root length (c) of Pendolino olive cuttings. Means with the same letter are not significantly different. .

Generally, NO promoted rooting up to 100 µM, after which higher concentrations were less effective or even inhibitory. The trend indicates a clear optimum for rooting percentage at 100 µM, with both lower and higher concentrations producing less favourable results (Fig. 1). These results are in line with Abu-Abied *et al.* (2012), who reported that NO at 1000 mg L^{-1}, together with 500 mg L^{-1} K-IBA, stimulated root growth in several plants, such as *Eucalyptus grandis.* Additionally, NO at 100 µM promoted rooting in several plants, such as chrysanthemum (Liao *et al.*, 2010), *Camellia sinensis* (Kang *et al.*, 2018), and *Panax ginseng* (Tewari *et al.*, 2008). NO at 100 µM stimulates rice root formation via the homeostasis of auxin or cross-talk with other hormones, such as ethylene (Kushwaha *et al.*, 2019), or by converting applied IBA to IAA via an oxidation pathway, which enhanced auxin binding to its receptors and facilitates adventitious root formation (Gonin *et al.*, 2019). NO is regarded as a downstream messenger for the auxin, which induced adventurous root formation at 20 µM in marigold plants (Liao *et al.*, 2009; Li *et al.*, 2020; Dave *et al.*, 2012). Additionally, it enhances the accumulation of carbohydrates as an energy source for growing cuttings (Haissig *et al.*, 1986). Li *et al.* (2019) reported that NO at 300 µM improves rooting of mung bean plants. Moreover, 100 µM NO improved the germination of hypocotyl lengths in Arabidopsis and lettuce seedlings, as well as the internode lengths in potato plants. In *Vigna radiata* seeds treated with NO at 0.5, 1, 2.5 and 5 µM), Sharma *et al.* (2019) found that high NO (5 µM) levels inhibited root lignification and downregulate POD and PPO activity.

The data indicate that the optimum NO concentration for rooting of Pendolino olive cuttings is 100 µM. In contrast, the highest NO concentration, 8000 µM had an adverse effect on the rooting of Pendolino olive cuttings. These results were in agreement with previous results as they reported that low NO concentration had a positive effect than high concentration in the rooting of many herbaceous plant species such as *Hydrilla verticillata* (Wang *et al.*, 2010), *Hyssopus officinalis* (Sadat-Hosseini and Soleimani, 2024), chrysanthemum (Liao *et al.*, 2010), and marigold (Liao *et al.*, 2009). Additionally, a low NO concentration improved the rooting of a non-herbaceous cherry plant (Sarropoulou *et al.*, 2015). In rubber (*Hevea brasiliensis*) plants, NO from 20 to 200 µM improved the rooting percentage (Nayanakantha *et al.*, 2014). The optimum NO concentration varies according to NO concentration, genotype sensitivity (Sarropoulou *et al.*, 2015), and experimental conditions.

Biochemical analysis

Total phenols: Total phenols content (Fig. 2a) was affected by NO applications. The highest total phenol content was recorded at 0, 50, and 4000 µM NO. While NO recorded the lowest total phenols at 100, 1000 and 8000 µM. Generally, the highest rooting percent was found in the low total phenols content (30.61 mg g^{-1} FW) at 100 µM NO, while higher total phenols (33.67 mg g^{-1} FW) were found in the low rooting percent treatments.

The impact of phenol content on rooting is significant as it can stimulate the rooting of cuttings in some species, such as olive (Rashedy, 2022), while it improves rooting in pomegranate

cuttings, which are regarded as easy to root (Fayek *et al.*, 2022), on the other hand, it restricts the rooting of some cuttings like magnolia cuttings (Wojtania *et al.*, 2019) and *Conocarpus erectus* (Abdel-Rahman *et al.*, 2020). At the same time, higher phenolic compounds increased rooting of terminal 'Manzanillo' cuttings (Abdel-Mohsen and Rashedy, 2023). The impact of specific phenol compounds on rooting luteolin-7-glycoside, rutin, tyrosol, oleuropein, diphenols, and total flavanols stimulates rooting of 'Kalamata' (Osterc *et al.*, 2009; Denaxa *et al.*, 2021). Also, Denaxa *et al.* (2021) reported that chromogenic acid, rutin, and quercetin have an important effect in the rooting of 'Arbe-quina' cuttings. Phenolic compounds, which have close relationships with enzymes related to auxin transport and metabolism (Martins *et al.*, 2022).

Total indoles: Total indoles (Fig. 2b) were not affected significantly by NO concentrations. These changes in total indoles may occur immediately after IBA application for 3 days, while the samples were taken 7 days after planting. High total indoles content was found positively related with high rooting % in olive cuttings (Rashedy, 2022; Abdel-Mohsen and Rashedy, 2023).

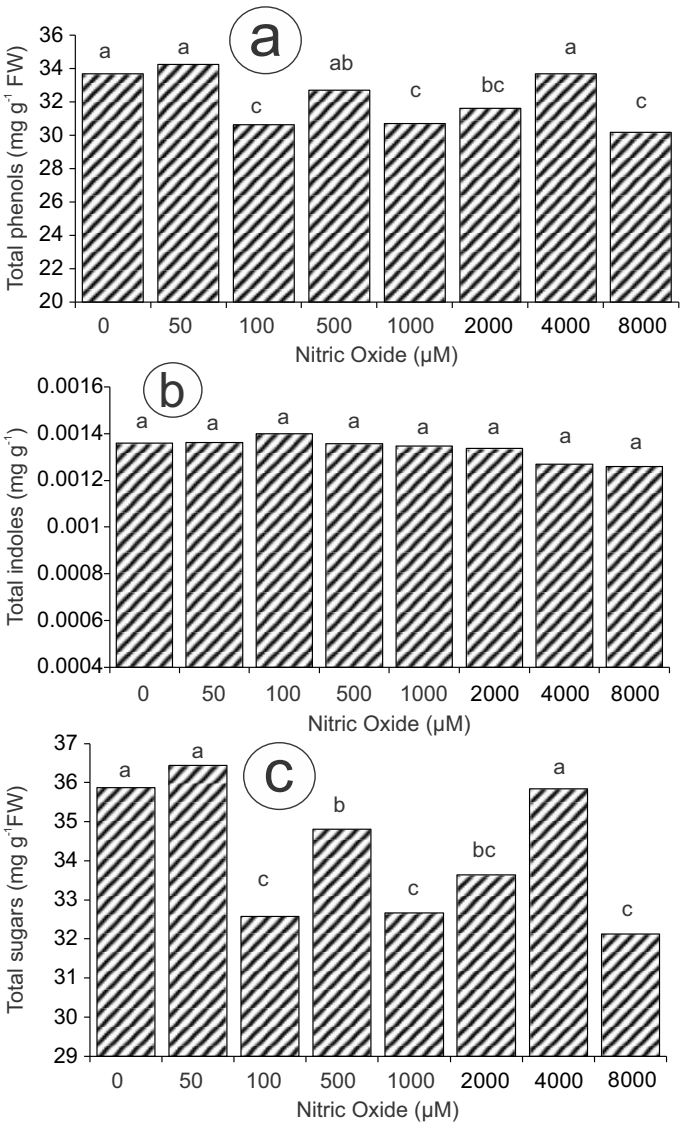

Fig. 2. Effect of different NO concentrations (0, 50, 100, 500, 1000, 2000, 4000, 8000 µM) on total phenols (a), total indoles (b), and total sugar content (c) of Pendolino olive cuttings. Means with the same letter are not significantly different.

Total sugar content: Total sugars (Fig. 2c) exhibited an unstable trend with increasing NO concentrations. Meanwhile, the highest rooting percentage was found in 100 µM NO. The lowest rooting was observed at 0, 50, 8000, and 4000 µM NO, which corresponded to higher total sugar content, except for 8000 µM NO, where the low sugar content may have resulted from the consumption, depletion, and utilization of sugars during the rooting process.

These results were similar to those of Abdel-Mohsen and Rashedy (2023), who reported lower sugars content of terminal Manzanillo olive cuttings accompanied by a higher rooting percentage. Also, Rashedy (2016) reported that a depletion of sugars was correlated with higher grafting success. Moreover, coratina olive cuttings treated with natural extracts, such as willow shoot and willow leaf extract, recorded the highest total sugars, accompanied by a low rooting percentage (Rashedy *et al.,* 2022).

Enzyme activity: Treatments with NO at 2000 and 100, 500, and 2000 µM recorded the highest CAT (Table 1). NO at 50 µM, followed by 8000, 0, and 1000 µM, showed the lowest CAT. There are no stable trends due to the high variation in NO concentration. Additionally, a higher rooting percentage was observed at 100 µM NO, followed by 1000 and 2000 µM, accompanied by high CAT activity, except at 2000 µM NO. POD recorded the lowest significant values at the low NO concentration (0, 50 µM) then it recorded the highest significant values at 100 followed by 500 µM NO then it reduced (Table 1). Higher POD accompanied with higher rooting % in NO at 100 µM while the lowest values of POD accompanied with the lowest values in control and then NO at 50µM.

Table 1. Effect of different NO concentrations on CAT, POD, PPO and IAAox activity of Pendolino olive cuttings

NO concentration	CAT	POD	PPO	IAAox
0 µM	3.420c	17.93d	12.79d	14.85b
50 µM	4.020b	17.87d	14.19cd	17.13a
100 µM	5.200a	33.21a	24.11a	8.307d
500 µM	4.733a	28.18b	11.19e	14.66bc
1000 µM	4.193b	21.74c	19.78b	5.69e
2000 µM	5.080a	21.87c	14.92c	6.773de
4000 µM	4.007b	27.99b	15.00c	12.96c
8000 µM	3.733bc	26.91b	14.04cd	14.9b

Means with the same letter are not significantly different (P=0.05).

Overall, PPO activity (Table 1) was similar to POD, as it recorded the lowest significant values at low NO concentrations, then increased, and then decreased. The highest PPO accompanied the highest rooting percentage at 100 µM NO, while the lowest rooting percentage was accompanied by the lowest PPO at the control, followed by NO at 50 and 8000 µM NO. IAAox trend was opposite to CAT, POD, PPO, since it recorded the highest significant values at the low NO concentrations (0, 50 µM), then it decreased significantly at (100 µM). After that, it increased slowly with increasing NO concentrations (Table 1). Generally, the low IAAox activity (8.31) was accompanied by the highest rooting percentage at 100 µM NO, followed by 1000 and 2000 µM NO. On the contrary, the lowest rooting percent was recorded in the control and NO at 50 and 8000 µM, which recorded the lowest rooting percent.

Generally, the highest enzyme activity was observed at 100 µM NO (Table 1). However, higher and lower NO concentrations

resulted in lower enzyme activity, which was accompanied by a lower rooting process. Additionally, the most active enzymes were POD and PPO.

These findings are in agreement with Chen *et al.* (2024), as they reported a significant positive relationship between CAT and POD activity and rooting of tea cuttings. Additionally, they found a decrease in IAAox activity and an increase in POD activity during the first root stages. In this study, CAT and PPO were increased by 21.2% and 69.9%, respectively, compared to the control (0 µM NO + 4000 ppm IBA).

The findings indicated that decreasing IAAox by 51.5% in the highest rooting treatments (100 µM NO) compared to the control. This may be due to the role of NO in extending auxin bioavailability for primordia initiation (homeostasis).

Additionally, the findings were in agreement with Wei *et al.* (2023), who reported that changes in POD activity could be used as biochemical markers for rooting stages in several plant species. In this respect, the increase in POD activity was 85.8%, which was the highest enzyme activity observed with the rooting treatment (100 µM NO) compared to the control (0 µM NO + 4000 ppm IBA).

Moreover, PPO catalyzes the condensation of phenolic compounds and IAA to form IAA-phenolic acid complexes, promoting root formation (Cao *et al.*, 2008). Additionally, PPO is crucial for root induction, and its activity increases at the early stage of rooting (Wei *et al.*, 2023). Our findings indicated that PPO increased by 69.9% in the highest rooting treatment (100 µM NO) compared to the control (0 µM NO + 4000 ppm IBA). Successful rooting depends on endogenous root promoters, such as enzymes, phenols, and sugar content. Raising promoters and lowering inhibitors resulted in higher rooting percentages. The phenol effect results from concentration and type. NO negatively reduces ABA, which may restrict root formation (Wang *et al.*, 2015).

Currently, the data on the impact of nitric oxide (NO) on rooting of olive cuttings is unavailable. The available literature has mainly dedicated its studies to herbaceous plants, including *Panax ginseng* (Tewari *et al.*, 2008), *Arabidopsis* (Shi *et al.*, 2014), chrysanthemum (Liao *et al.*, 2010), marigold (Liao *et al.*, 2009; 2011), cucumber (Xu *et al.*, 2017; Li *et al.*, 2020) and mung bean (Li *et al.*, 2019). Not much research has been done regarding non-herbaceous plants, as well (*Camellia sinensis*) (Kang *et al.*, 2018). Finally, the positive or negative consequences of NO are based on the concentration of it, its genotype sensitivity (Sarropoulou *et al.*, 2015), and the experimental circumstances.

Generally, NO does not affect the rooting of cuttings by mediating hormone signaling pathways that stimulate cell division and elongation at low NO concentrations (Forde, 2002). NO at low concentrations may promote plant growth by reducing cell wall lignification and accelerating cell expansion (Wang *et al.*, 2010). On the other hand, high NO concentrations may promote membrane leakage due to oxidative stress which inhibits plant growth (Wang *et al.*, 2010).

NO at low concentrations has the dual role of functioning as a signal molecule. In contrast, at high concentrations, it acts as a stress molecule, associated with damage to macromolecules caused by protein nitration (Corpas *et al.*, 2015). This causes severe damage to proteins and membranes, thereby restricting cellular functions (Yamasaki and Cohen, 2016).

Rooting response to nitric oxide (NO) depends strongly on its concentration. Rooting in Hyssopus officinalis enhanced with NO levels up to 15 µM but decreased at 20 µM (Sadat-Hosseini and Soleimani, 2024). Similarly, greater NO levels (40-50 µM) decreased leaf glucose content (Sarropoulou *et al.*, 2015). In marigold, NO at 10-200 µM increased root length and number, but 1000 µM hindered rooting (Liao *et al.*, 2009). Chrysanthemum cuttings showed maximum rooting at 100 µM, while higher doses (200–500 µM) reduced rooting traits (Liao *et al.*, 2010). In rubber (*Hevea brasiliensis*), moderate levels of 20–200 µM were more effective than higher concentrations (Nayanakantha *et al.*, 2014). Overall, these studies indicate that moderate NO concentrations stimulate rooting, whereas excessive levels become inhibitory.

The study showed that NO had beneficial effect on the rooting of Pendolino olive cuttings by modulating activities of key biochemical and enzymatic parameters. The treatment with 100 µM was most effective enhancing root percentage, root number and root length and resulted in higher activities of catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO) alongwith lower activity of indole-3-acetic acid oxidase IAAox. This result indicate that NO at this concentration plays a role in promoting root initiation and development by increasing antioxidant enzyme activities, reducing inhibitory factors such as IAA oxidase and phenol accumulation, and keeping the auxin homeostasis. Screening of NO concentration further showed that concentrations above or below optimal levels were suboptimal or inhibitory to rooting, highlighting the need for well-suited NO dosages forsuccessful vegetative propagation of such recalcitrant cultivar as olive. Overall, the data presented here provide important information on the dual role of NO and its applications in signal transduction or associated with environmental stress responses that can be useful for improving rooting through olive and other woody species.

References

Abdel-Mohsen, M.AA. and A.A. Rashedy, 2023. Stock plant etiolation reduces rooting of sub-terminal olive cuttings by reducing total sugars, IAA, indole/phenol ratio and IAA/GA ratio. *Acta Physiol. Plant*., 45: 104.

Abdel-Rahman, S.S.A., E.Y. Abdul-Hafeez and A.M.M. Saleh, 2020. Improving rooting and growth of *Conocarpus erectus* stem cuttings using indole-3-butyric acid (IBA) and some biostimulants. *Sci. J. Flowers Ornam. Plants,* 7(2): 109-29.

Abu-Abied, M., D. Szwerdszarf, I. Mordehaev, A. Levy, E. Belausov, Y. Yaniv, S. Uliel, M. Katzenellenbogen, J. Riov and R. Ophir, 2012. Microarray analysis revealed upregulation of nitrate reductase in juvenile cuttings of *Eucalyptus grandis*, which correlated with increased nitric oxide production and adventitious root formation. *Plant J.*, 71: 787-799.

Aebi, H. 1984. Catalase *in vitro*. *Methods in Enzymology*, 105: 121-126.

Al-Allaf, A.H.E. 2009, Auxins application on propagation of olive cv. Chemlali by semi-hardwood cuttings. *Mesop. J. Agric.,* 37(4): 1-9.

Al-Hamdani, Kh.A.S. and El.S.Kh. Mohammed, 2017. Effect of cutting length and indole butyric acid and hydrogen peroxide on two rooting Ba'shiqah and Menzinelloa olive cultivars *Olea europea* L. *Kirkuk Univ. J. Agric. Sci.*, 8(4): 79-96.

Beligni, M.V. and L. Lamattina, 2000. Nitric oxide stimulates seed germination and de-etiolation and inhibits hypocotyl elongation, three light-inducible responses in plants. *Planta,* 210: 215-221.

Cao, B.H., H.J. Hu, D.P. Zhang and X.D. Zhu, 2008. Rooting capacity and correlative enzyme activities of hardwood cuttings of mulberry. *Canye Kexue*, 34(1): 96-100.

Chance, B. and A.C. Maehly, 1955. Assay of catalases and peroxidases. *Methods Enzymol.*, 2: 764-775.

Chen, W., T. Niu, W. Lian, T. Ye, Q. Sun and J. Zhang, 2024. Involvement of endogenous IAA and ABA in the regulation of arbuscular mycorrhizal fungus on rooting of tea plant (*Camellia sinensis* L.) cuttings. *BMC Plant Biology,* 24: 1266

Corpas, F.J. and J.B. Barroso, 2015. Review functions of nitric oxide (NO) in roots during development and under adverse stress conditions. *Plants,* 4: 240-252.

Dave, A. and I.A. Graham 2012, Oxylipin signaling: A distinct role for the jasmonic acid precursor cis-(+)-12-oxo-phytodienoic acid (cis-OPDA). *Front. Plant Sci.,* 3, 42.

Denaxa, N.K., S.N. Vemmos and P.A. Roussos, 2021. Shoot girdling improves rooting performance of kalamata olive cuttings by upregulating carbohydrates, polyamines and phenolic compounds. *Agriculture*, London, 11: p71.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for the determination of sugars and related substances. *J. Anal. Chem.,* 28: 350-356.

Eid, A.M.H., S.A. Nomier, M.M. Ibrahim and M.M. Gad, 2018. Effect of some natural extracts, indolebutyric acid and naphthalene acetic acid on rooting of picual olive cuttings. *Zagazig J. Agric. Res.*, 45(1): 119-136.

Fabbri, A., G. Bartolini, M. Lambardi and S. Kailis, 2004. Olive propagation manual. Landlinks Press, Collingwood, pp 141.

FAO, 2023. Food and Agriculture Organization. www.fao.org/faostat/en/#data/QCL. Access June 2025.

Fayek, M.A., A.E. Mohamed and A.A. Rashedy, 2022. Deficit irrigation effects on five pomegranate cultivars' water use efficiency and biochemical parameters. *J. Appl. Hortic.,* 24(1): 27-32.

Forde, B.G. 2002. Local and long-range signaling pathways regulating plant responses to nitrate. *Annu. Rev. Plant Biol.* 53, 203-224.

Freed, R., S.P. Eisensmith, S. Goetz, D. Reicosky, V.M. Smail and P. Wollberg, 1990. MSTAT-C a microcomputer program for the design, management and analysis of agronomic research experiments. Michigan: Michigan State University, 1990. Disponível em: https//www.msu.edu/~freed/disks.htm.

Gonin, M., V. Bergougnoux, T.D. Nguyen, P. Gantet and A. Champion, 2019. What makes adventitious roots? *Plants*, 8: 240.

Gouvinhas, I., N. Machado, C. Sobreira, R. Domínguez-Perles, S. Gomes, E. Rosa and A.I.R.N.A. Barros, 2017. Critical review on the significance of olive phytochemicals in plant physiology and human health. *Molecules*, 22: 1986.

Haissig, B.E. 1986. Metabolic processes in adventitious rooting of cuttings. In: *New Root Formation in Plants* and *Cuttings.* Jackson, M.B., Ed.; Springer: Dordrecht, The Netherlands, pp. 141-189.

Hussein, B.A., Y.A.R. Goran and M.Q. Khurshid, 2017. Effect of different concentrations of IBA on rooting ability and shooting in olive (*Olea europaea* L., cv. Dgel) cuttings. International Conference and Workshop on Basic and Applied Sciences, March 18th-19th 2017, ErbilKRG-IRAQ 1-11.

IOC, 2025. International Olive Council https//www.internationaloliveoil.org/world-market-of-olive-oil-and-table-olives-data-from-december-2024/

Kang, W., L.Y. Wang, R. Li, C.C. Zhang, L.Y. Wu and H.L. Li, H. Cheng, 2018. Endogenous nitric oxide and hydrogen peroxide detection in indole-3-butyric acid-induced adventitious root formation in *Camellia sinensis*. *J. Integr. Agric.,*17: 2273-2280.

Kushwaha, B.K., S. Singh, D.K. Tripathi, S. Sharma, S.M. Prasad, D.K. Chauhan, V. Kumar and V.P. Singh, 2019. New adventitious root formation and primary root biomass accumulation are regulated by nitric oxide and reactive oxygen species in rice seedlings under arsenate stress. *J. Hazard. Mater*., 361: 134-140.

Larsen, P., A. Harbo, S. Klungron and T.A. Ashein, 1962. On the biosynthesis of some indole compounds in *Acetobacter Xylinum*. *Physiol. Plant*., 5: 552-565.

Li, S-W, Y. Li, Y. Leng, X-Y and Yan-Hua Maa, 2019, Nitric oxide donor improves adventitious rooting in mung bean hypocotyl cuttings exposed to cadmium and osmotic stresses. *Environ. Exp. Bot*., 164: 114-123.

Li, Y.T., Y. Wu, W.B Liao, L.L. Hu, M.M. Dawuda, X. Jin, Z.Q.Tang, J.J.Yang and J.H. Yu, 2020. Nitric oxide is involved in the brassinolide-induced adventitious root development in cucumber. *BMC Plant Biol.*, 20: 102.

Liao, W., H. Xiao and M. Zhang, 2009. Role and relationship of nitric oxide and hydrogen peroxide in adventitious root development of marigold. *Acta Physiol. Plant.*, 31: 1279-1289.

Liao, W., M. Zhang and J. Yu, 2013. Role of nitric oxide in delaying senescence of cut rose flowers and its interaction with ethylene. *Sci. Hortic.,*155: 30-38.

Liao, W., G. Huang, J. Yu, M. Zhang and X. Shi, 2011. Nitric oxide and hydrogen peroxide are involved in indole-3-butyric acid-induced adventitious root development in marigold. *J. Hortic. Sci. Biotechnol.*, 86: 159-165.

Liao, W.B., H.L. Xiao and M.-L. Zhang, 2010. Effect of nitric oxide and hydrogen peroxide on adventitious root development from cuttings of ground-cover chrysanthemum and associated biochemical changes. *J. Plant Growth Regul.*, 29: 338-348.

Mahadevan, A. and R. Sridhar, 1982. *Methods in Physiological Plant Pathology.* Sivakami Publications, Madras, India.

Martins, M., A.F.G. Gomes, É.M. Da Silva, D.F. Da Silva, P.M. Peche, T.A. Magalhães and R. Pio, 2022. Effects of anatomical structures and phenolic compound deposition on the rooting of olive cuttings, *Rhizosphere*, 23: 100557.

Mayer, A.M., E. Harel and R.B. Shaul, 1965. Assay of catechol oxidase, a PPO. *Phytochem*., 5(4): 783-789.

Mohammed, A.A. 2021. Application of different concentrations of licorice and willow extracts as rooting stimulator in hardwood cuttings of olive (*Olea europaea* L.). *Int. J. Environ, Agric. and Biotech.*, 6 (6): 58-63.

Nayanakantha, N.M.C, P.D. Pathirana, A.M.W.K. Senevirathna and P. Seneviratne, 2014. Exogenous nitric oxide donor sodium nitroprusside ameliorates root architecture and growth performance in young budding polybagged plants of rubber (*Hevea brasiliensis). J. Rubber Res. Inst. Sri Lanka,* 94: 9-24.

Neill, S., R. Barros, J. Bright, R. Desikan, J. Hancock, J. Harrison, P. Morris, D. Ribeiro and I. Wilson, 2008. Nitric oxide, stomatal closure and abiotic stress. *J. Exp. Bot.,* 59: 165-176.

Osterc, G., M. Stefancic and F. Stampar, 2009. Juvenile stock plant material enhances root development through higher endogenous auxin levels. *Acta Physiol. Plant.,* 31: 899- 903.

Rashedy, A.A. 2016. Effect of pre-grafting incubation and grafted cuttings position on grape grafting success. *Egypt. J. Hort*., 43(2): 225-240.

Rashedy, A.A. 2022, Impact of some natural extracts on rooting performance of coratina olive cuttings. *Rev. Bras. Frutic.*, 44(5) e-972.

Rashedy, A.A., W.A.M. Eldeeb and H.H. Hamed, 2021. Antioxidant procedure improve olive cuttings rooting during the cool season. *Egypt. J. Hortic.*, 48(2): 267-75.

Rockel, P., F. Strube, A. Rockel, J. Wildt and W.M. Kaiser, 2002. Regulation of nitric oxide (NO) production by plant nitrate reductase *in vivo* and *in vitro*. *J. Exp. Bot.*, 53: 103-110.

Rockel, P. and W.M. Kaiser, 2002. NO Production in plants: Nitrate reductase versus nitric oxide synthase. In: Progress in Botany. vol 63. Springer, Berlin, Heidelberg. Esser, K., Lüttge, U., Beyschlag, W., Hellwig, F. (eds)

Sadat-Hosseini, M. and A. Soleimani, 2024. Callus Induction, Shoot and Root Regeneration in *Hyssopus officinalis* Using Sodium Nitroprusside and Plant Growth Regulators. *J. Medicinal Plants and By-products*, 13(4): 932-939.

Sarropoulou, V., K. Dimassi-Theriou and I. Therios 2015. Effect of sodium nitroprusside on micropropagation and biochemical parameters of CAB-6P and Gisela 6 cherry rootstocks. *Turk. J. Biol.* 39(4): 595-610.

Sharma, A., B. Shahzad, A. Rehman, R. Bhardwaj, M. Landi and B. Zheng, 2019. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules*, 24: 1-22.

Shi, H., Y. Tiantian, J. Zhu and Z. Chan, 2014. Constitutive production of nitric oxide leads to enhanced drought stress resistance and extensive transcriptional reprogramming in *Arabidopsis. J. Exp. Bot.*, 65(15): 4119-4131.

Skiba, U., K.A. Smith and D. Fowler, 1993. Nitrification and denitrification as sources of nitric oxide and nitrous oxide in a sandy loam soil. *Soil Biol Biochem.,* 25: 1527-1536.

Snedecor, W. and W.G. Cochran, 1989, *Statistical Methods*. 8th. Iowa: Iowa State University Press, 1989. 503p.

Tewari, R.K., E.J. Hahn and K.Y. Paek, 2008. Function of nitric oxide and superoxide anion in the adventitious root development and antioxidant defence in *Panax ginseng*. *Plant Cell Rep*., 27: 563-573.

Wang Y.Q., A. Feechan, B.W. Yun, R. Shafiei, A. Hofmann, P. Taylor, P. Xue, F.Q. Yang, Z.S. Xie, J.A. Pallas, C.C. Chu and G.J. Loake, 2009. S-nitrosylation of AtSABP3 antagonizes the expression of plant immunity. *J. Biol. Chem.,* 284(4): 2131-2137.

Wang, P., Y. Du, Y.J. Hou, Y. Zhao, C.C. Hsu, F. Yuan, X. Zhu, W.A. Tao, C.P. Song and J.K. Zhu, 2015. Nitric oxide negatively regulates abscisic acid signaling in guard cells by S-nitrosylation of OST1. *Proc Natl Acad Sci USA*, 112(2): 613-618.

Wang, H., S. Zhang, W. Zhang, C. Wei and P. Wang, 2010. Effects of nitric oxide on the growth and antioxidant response of submerged plants *Hydrilla verticillata* (L.f.) *Royle. Afr. J. Biotechnol.,* 9: 7470-7476.

Wei, P., Y. Lv, Q. Guang, J. Han, Y.F. Wang, X.W. Wang and L. Song, 2023. ChIFNα regulates adventitious root development in *Lotus japonicus* via an auxin-mediated pathway. *Plant Signal Behav.,*18(1): 2218670.

Wojtania, A., E. Skrzypek and A. Marasek-Ciolakowska, 2019. Soluble sugar, starch and phenolic status during rooting of easy and difficult-to-root magnolia cultivars. *Plant Cell, Tissue and Organ Culture,* 136: 499-510.

Xu, X.T., X. Jin, W.B. Liao, M.M. Dawuda, X.P. Li, M. Wang, L.J. Niu, P.J. Ren and Y.C. Zhu, 2017. Nitric oxide is involved in ethylene-induced adventitious root development in cucumber (*Cucumis sativus* L.) explants. *Sci. Hortic.,* 215: 65-71.

Yamasaki, H. and M.F. Cohen, 2016. Biological consilience of hydrogen sulfide and nitric oxide in plants: Gases of primordial earth linking plant, microbial and animal physiologies. *Nitric Oxide*, 55: 91-100

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