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EFFECT OF SALICYLIC ACID IN INHIBITING FUNGAL CONTAMINATION IN *IN VITRO* CULTURES OF DATE PALM (*PHOENIX DACTYLIFERA* L.) AND ENHANCING EMBRYOGENESIS AND PLANTLET DEVELOPMENT

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ABSTRACT

In this study, we evaluated the role of salicylic acid in diminishing fungal contamination in *in vitro* cultures of date palm and the effect of salicylic acid on the regeneration of somatic embryos from callus formed on shoot tips. The most prevalent fungi were *Alternaria alternata* (37%), *Fusarium solani* (25%), *Aspergillus fumigatus* (18%), and *Penicillium expansum* (6%). Salicylic acid limited and at higher concentrations retarded mycelial growth using potato dextrose agar. Salicylic acid at concentrations of 1.5 and 2.0 mM added to MS medium with 2iP and NAA significantly increased the embryogenesis rate of calli explants to 64.9% and 56.7%, respectively, compared with the control (12.3%). Salicylic acid also increased plantlet development from embryos by about 27% compared with the control. Salicylic acid caused better shoot and root growth and increased chlorophyll content. The results showed that the addition of salicylic acid at 1.5 mM to the MS medium resulted in a significant increase in the concentrations of IAA and ABA, as well as a decrease in the concentration of IBA in leaves.

Key words: initial cultures, somatic embryogenesis, plantlets development, content of growth regulators

INTRODUCTION

Microbial contamination of date palm cultures can occur at any stage of the *in vitro* culture process. It may be derived from endogenous tissues of the initial explants or introduced during processing (Al-Mussawii 2010). Fungal contamination accounts for 3–15% of *in vitro* losses. A previous study by Jassim et al. (2021) identified the most common fungal contaminants in *in vitro* date palm culture as *Alternaria alternate* 45%, *Fusarium* spp. (32%), *Aspergillus* spp. (17%), and *Penicillium* spp. (6%).

Salicylic acid (SA) is a phenol that affects fruit ripening, senescence, growth, and quality. It also activates the body's defense system against local and systemic infections (Chen et al. 2020). Studies have shown that applying SA can improve shoot and root development of treated plants. According to Sanaa et al. (2001), SA promotes cell division, metabolism of natural hormones IAA, GA_3, and cytokinins, absorption of nutrients and water, photosynthesis, production of plant pigments, and uptake of most organic nutrients. The process by which SA promotes growth may also be linked to variations in other growth hormone levels. According to Santner et al. (2009), SA can increase cell metabolism, reduce lipid oxidation, eliminate excess reactive oxygen species from cells, strengthen the plant's antioxidant defense, and ultimately reduce the effects of salt stress. These findings highlight the increasing role of SA in phytotoxicology (Guo et al. 2019).

SA is crucial in activating plant disease resistance mechanisms, such as systemic acquired resistance (Ding et al. 2018). A study by Al-Mayahi (2016) showed that the applying 50 $mg \cdot L^{-1}$ SA to date palms grown *in vitro* led to increased shoot growth and the chlorophyll content enhanced the activity of antioxidant enzymes SOD and APX.

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In a study by Suhaib et al. (2018), it was discovered that under normal conditions, the application of SA at concentrations of 0.25 mM and 0.50 mM resulted in increased chlorophyll content compared to the control group. Additionally, these concentrations boosted root length by 49.5% and 42.9%, and shoot length by 15.8% and 12.5%, respectively, compared to the control. These results are consistent with those of Jazi et al. (2011) who found that the plant developed more shoots when *Brassica napus* was exposed to SA under heavy metal stress. Turkyilmaz (2012) discovered that applying SA in wheat plants increased the shoot length.

SA has been identified as regulating several elements of leaf physiology. It can control the quantity of photosynthetic pigment, alter the activity of carbon-absorbing photosynthetic enzymes, increase the efficiency of the photosynthetic mechanism, and regulate stomatal activity. Similar results were obtained by Amin et al. (2008) when they sprayed 100 $mg \cdot L^{-1}$ SA on the leaves of wheat plants (*Triticum aestivum* L.), improving growth parameters, photosynthetic pigment content, and productivity. In addition, SA was shown to increase the proportion of total carbohydrates in wheat grains compared to control plants. SA has also been shown to have favorable effects on cowpea plants, including an increase in the number of productive tillers per plant, as well as improvements in plant height, productivity, and photosynthetic pigment content (El-Taher et al. 2022).

Several investigations have shown that SA has an effect on microorganisms. It activates plant disease resistance mechanisms such as pattern-triggered immunity, effector-triggered immunity, and systemic acquired resistance (Ding et al. 2018; Radojičić et al. 2018). Furthermore, previous studies have shown that SA demonstrates antifungal activity against *Fusarium oxysporum* and *Penicillium expansum*, preventing hyphal development and spore germination (Wu et al. 2008; da Rocha Neto et al. 2015). According to Zamani et al. (2019), SA at a dose of 4 mM inhibited conidia germination and mycelium growth of the fungus *Zymoseptoria tritici* when added to yeast malt dextrose agar. According to Klessig et al. (2016), SA primarily activates adenosine monophosphate-protein kinase, a crucial regulator of cell development and metabolism. De Vleesschauwer et al. (2013) state that plant hormones are crucial for regulating plant growth and development and prolonging plant life. These hormones are essential for transmitting signaling pathways and facilitating cross-talk, which is necessary for many biological processes. It also controls physiological and biochemical processes throughout a plant's life cycle, particularly in response to biotic and abiotic stress (Karuppaiah et al. 2003).

In this study, the effect of SA on the initiation of *in vitro* culture of 'Barhi' date palm from shoot tips derived from offshoots was investigated. The scope of the study included fungal contamination, embryogenesis, shoot development, and chlorophyll content, as well as IAA, ABA, and IBA in leaves.

MATERIALS AND METHODS

Establishment of in vitro cultures

The study began by selecting healthy-looking young offshoots from the mother date palm trees. Offshoots 50–70 cm long, 3–4-year-old and weighing 4–8 kg, were detached from the mother trees and transferred to the laboratory. Adventitious roots, fibrous sheath, and leaves were removed to reveal the shoot tips. Explants were sterilized with 70% ethanol for 1 minute and 2.5% sodium hypochlorite for 20 minutes and then rinsed three times with sterile distilled water. Apical buds (8–12 mm long) were sectioned longitudinally into four parts. Explants were transferred to MS basal medium (Murashige & Skoog 1962) supplemented with 3 $mg \cdot L^{-1}$ 6-(γ,γ-dimethylallylamino)purine (2iP), 30 $mg \cdot L^{-1}$ naphthylacetic acid (NAA), 1.5 $g \cdot L^{-1}$ activated charcoal (AC) and solidified with agar-agar at 7.0 $g \cdot L^{-1}$. SA was added to the MS medium at four concentrations (1.0, 1.5, 2.0, and 2.5 mM). Explants were kept in complete darkness at 27 ± 2 °C. They were transferred to fresh media of the same composition every 6 weeks until callus was initiated (two subcultures).

Callus was excised and cultured on MS basal medium as above, except for AC (0.5 m·L^{-1}). Cultures were maintained at 25 ± 2 °C with cabinet lighting of 1500–2000 lx, cool-white fluorescent lamps, and a photoperiod of 16 h light/8 h dark.

The results of the experiments were evaluated 12 weeks after callus separation on MS medium supplemented with SA concentrations (0.0, 1.0, 1.5, and 2.0 mM). There were twelve replicates of each treatment and three calli per jar. We looked at the percentage of embryogenic callus and shoot growth. The percentage of fungal contamination and callus browning was also monitored. The percentage of calli transformed into shoots, the number of proliferated shoots, and the length of shoots and roots were also assessed. Finally, total chlorophyll content in leaves was estimated according to Barnes et al. (1992) as well as the contents of IAA, IBA, and ABA.

Samples of 1 gram of new fresh bulk of leaves were homogenized and ground in liquid nitrogen. Extraction was then carried out using 30 mL of 80% cold methanol in the dark at 4 °C. The concentrate was centrifuged at 5,000 rpm for 15 minutes at 4 °C, and the supernatant was collected in a microcentrifuge tube. The residue was resuspended in 15 mL of extraction solvent and centrifuged, and the supernatant was combined with the microcentrifuge tube. The methanol was separated and combined with 10 ml of methanol in a rotary evaporator. Abscisic acid (ABA) was measured against a standard reference of ABA (Kamboj et al. 1999). The liquid chromatography method of Kelen et al. (2004) with the Shimadzu HPLC/UHPLC/SFC system (Japan) was used to measure indoleacetic acid (IAA) and indolebutyric acid (IBA). The mobile phase for separation was acetonitrile-water containing 30 mM phosphoric acid, pH 4.0, with a flow rate of 0.8 µL min^{-1}. Each extraction involved three separate injections. The concentration of sample solutions were detected at wavelengths of 208 nm, 265 nm, and 280 nm for IAA, ABA, and IBA, respectively.

Isolation and identification of fungi from contaminated cultures

Fungi in culture tubes were isolated using potato dextrose agar (PDA) medium. After 4–7 days of incubation, single-spore cultures were used to purify fungal isolates and transfer them to new medium. The fungi were then classified based on their morphological properties, including colony morphology, color, conidia, conidiophore and chlamydospore formation. These characteristics were observed under a light microscope after staining with methylene blue dissolved in lactophenol (Olympus CX21, Japan) (Barnett & Hunter 1972; Domsch et al. 1993). The frequency of fungal contamination was calculated in percentages.

Molecular identification was performed according to Jassim and Ahmed (2024). Nucleotide sequences were aligned and compared with sequences of fungal isolates available in the NCBI database using the basic local alignment search tool (BLAST). Phylogenetic analysis of all fungal nucleotide sequences was conducted using MEGA 6.

Effect of salicylic acid on isolated fungi

To 20 ml of potato dextrose agar (PDA) medium, 1.0, 1.5, 2.0, and 2.5 mM SA was added and poured into each Petri dish (9 cm). Five-mm disks of 5-day-old cultures of each test fungus were taken from the edges of the fungal contamination plates, placed in the center of the Petri dishes, and incubated at 28 ± 2 °C. PDA medium without SA served as control. For each treatment, five replicates were evaluated. Observations were recorded every 12 hours and after the completion of growth of the mycelium colonies in the control (9 cm). The diameter of the mycelium growth colony for each fungus was measured in millimeters. The percentage of growth inhibition (GI) was calculated using the following formula: GI (%) = (dc − dt/dc) × 100; where dc is the growth rate of mycelium colonies in the control and dt is the growth rate of mycelium colonies in the SA concentration treatments (Singh & Tripathi 1999).

Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA). Statistically significant differences between treatments were detected using the least significant difference (LSD) method and a p value of 0.05. Statistical analysis was conducted using the SPSS software package.

RESULTS

Isolation and molecular identification of fungi from contaminated cultures

The most frequently isolated fungi in the initial *in vitro* cultures of date palm were *Alternaria alternata* – constituting approximately 37%, followed by *Fusarium solani* – 25%, *Aspergillus fumigatus* – 18%, and *Penicillium expansum* – approximately 6%. The genetic similarity between our fungi and reference isolates from the NCBI database was 99.5% for *A. alternata*, 99% for *F. solani*, 99% for *A. fumigatus*, and 100% for *P. expansum*.

The effect of salicylic acid on mycelium growth

The addition of SA to potato dextrose agar (PDA) media at concentrations of 1.0, 1.5, 2.0, and 2.5 mM decreased the mycelial growth of all fungi depending on the SA concentration. At concentrations of 1.5, 2.0, and 2.5 mM the growth of all fungal colonies was reduced by 100%, and after adding 1 mM SA to the PDA medium the growth of fungal colonies was reduced by 57.5% compared to the control without SA (Fig. 1).

Effect of salicylic acid on date palm explants

SA at concentrations ranging from 1.0 mM to 2.5 mM affected somatic embryogenesis formed on callus (Table 1). Media with 1–2 mM SA added reacted at a higher percentage (38.6–64.9% compared to 12% in the control). The highest percentage of regeneration (65 and 56) and developed shoots (20 and 18 per jar) were observed on media containing 1.5 mM and 2.0 mM SA (Fig. 2A). At a concentration of 2.5 mM SA, regeneration was delayed, callus did not develop and perished. After three months, the average length of the longest shoots on 1.5 mM and 2.0 mM SA media was 11.1 and 9.5 cm, respectively, and the length of the roots was 7.7 and 5.9 cm, respectively, while on the control medium these values were 6.3 and 3.3 cm, respectively (Fig. 2B).

The highest concentration of total chlorophyll was detected in leaves obtained on media supplemented with 1.5 and 2.0 mM SA. The mean values ranged from 6.2 to 6.3 $mg \cdot g^{-1}$ fresh weight (FW), while on the medium without SA addition (control) this value was 4.8 $mg \cdot g^{-1}$ FW (Table 2).

On media without SA, 18.3% of cultures were contaminated, whereas on media with 1 mM this was 6.2% and at 1.5 and 2.0 mM there was no contamination. Callus browning reactions were observed on the control medium – 14.7%, on the medium with 1.0 mM – 6.9%, and on the medium with 1.5 and 2.0 mM – 2.6% and 2.8%, respectively (Table 3).

The addition of SA to MS medium significantly affected the levels of plant hormones. The IAA content in leaves grown on SA medium increased by 29–50% compared with the control. ABA content was 44–82% higher after SA addition, while IBA concentration decreased in media with SA by 40–62% compared with the control (Table 4).

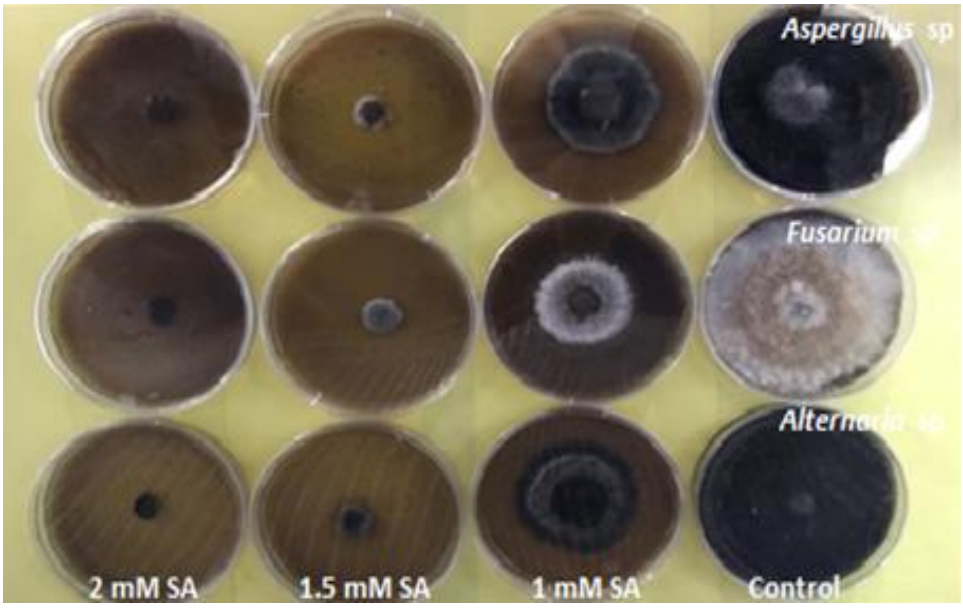

Figure 1. Effect of salicylic acid added to PDA medium on the growth of mycelia of *Aspergillus fumigatus*, *Fusarium solani*, and *Alternaria alternata*

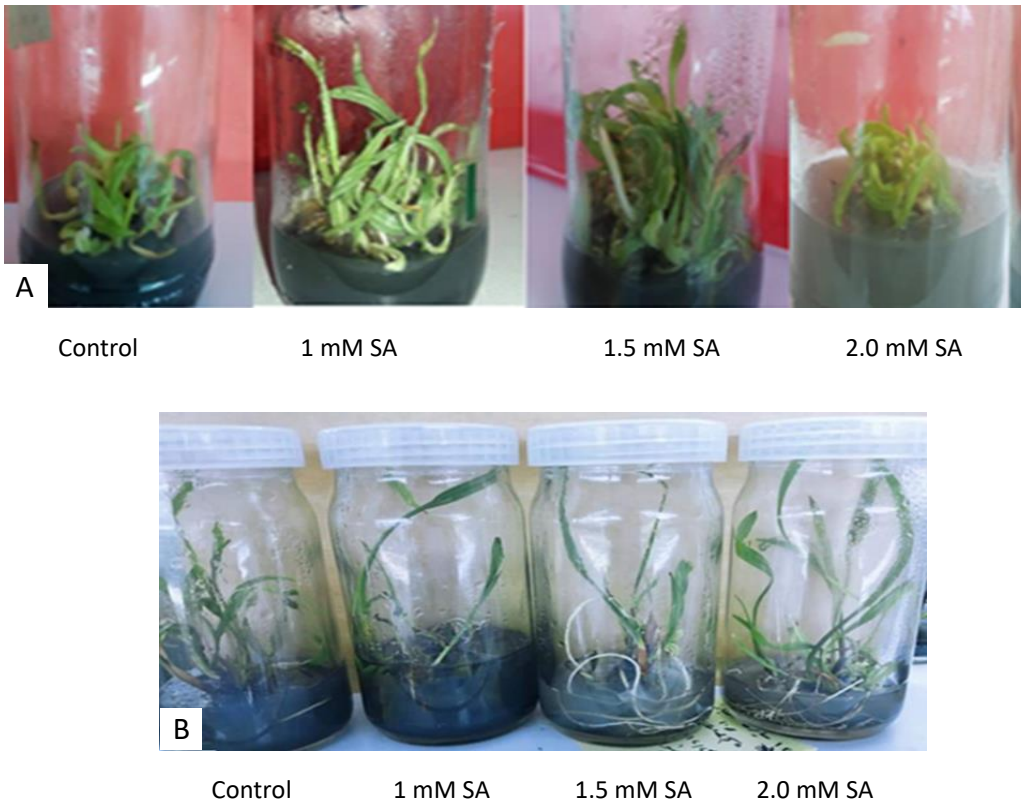

Figure 2. Effect of salicylic acid on shoot regeneration (A), shoot and root elongation (B) compared with the control (no SA added)

Table 1. Effect of salicylic acid supplemented to MS medium on the percentage of somatic embryogenesis and the number of shoots regenerated from embryos

Salicylic acid (mM)	Percentage of somatic embryogenesis	Average number of shoots per jar
0.0	12.3c ± 0.050	15.9b ± 0.04
1.0	38.6b ± 0.020	19.2b ± 0.04
1.5	64.9a ± 0.077	20.4a ± 0.031
2.0	56.7a ± 0.070	18.5a ± 0.03
2.5	0.9d ± 0.00	3.0c ± 0.00
LSD	8.55	1.07

Note: Values followed with the same letters do not differ significantly from each other at p= 0.05; LSD – least significant difference

Table 2. Effect of salicylic acid supplemented to MS medium on shoot and root length

Salicylic acid (mM)	Length of longest roots (cm)	Length of longest shoots (cm)	Total chlorophyll content in leaves ($mg \cdot g^{-1}$ FW)
0.0	3.3b ± 0.030	6.3b ± 0.020	4.75c ± 0.025
1.0	6.6a ± 0.037	7.8b ± 0.064	5.44b ± 0.040
1.5	7.7a ± 0.062	11.1a ± 0.200	6.27a ± 0.020
2.0	6.0a ± 0.034	9.5ab ± 0.125	6.17a ± 0.037
LSD 0.05	2.30	2.9	

Note: see Table 1

Table 3. Effect of salicylic acid supplemented to MS medium on percentage of fungal contamination and callus browning

Salicylic acid (mM)	Percentage of cultures with fungal contamination	Percentage of callus browning
0.0	18.3 ± 0.05	14.7b ± 0.08
1.0	6.2 ± 0.02	6.9c ± 0.02
1.5	0	2.6d ± 0.00
2.0	0	2.8e ± 0.00
LSD 0.05	-	1.57

Note: see Table 1

Table. 4 Effect of salicylic acid supplemented to MS medium on the concentration of IAA, ABA, and IBA

Salicylic acid (mM)	IAA	ABA	IBA ($\mu g \cdot g^{-1}$ FW)
0.0	0.038b ± 0.002	0.308d ± 0.006	5.364ba ± 0.004
1.0	0.052a ± 0.003	0.562a ± 0.007	3.228 ± 0.005
1.5	0.057aa ± 0.004	0.485b ± 0.004	2.062d ± 0.002
2.0	0.049 ± 0.004	0.443c ± 0.0023	3.058c ± 0.006
LSD 0.05	0.015	0.023	0.140

Note: see Table 1

DISCUSSION

In our study, the most frequent fungi contaminating initial date palm cultures were *A. fumigatus*, *F. solani*, *A. alternata*, and *P. expansum*, which is consistent with the results of other studies on different date palm genotypes (Abass 2013).

SA significantly inhibited the growth of mycelium of contaminating fungi. Our study showed that applying SA (1.5 and 2.0 mM) to MS medium eliminated fungal contamination. Qi et al. (2012) obtained similar results when studying the effect of SA on the fungus *F. graminearum* causing head blight in wheat. Their findings revealed that SA substantially affected the efficiency of conidia germination and mycelium growth; a concentration of 4 mM was adequate to prevent disease in susceptible and partially resistant cultivars. According to Zamani et al. (2019), exogenous administration of SA can effectively stop hyphal development, spore formation, and pathogenicity of *F. oxysporum*. Cowan (1999) proposed that the toxicity of SA to microorganisms is caused by oxidized molecules inhibiting enzyme function, perhaps through reactions with sulfhydryl groups. The number and location of hydroxyl groups in the phenol group play a significant role in their relative toxicity to pathogens. SA has an inhibitory effect on *F. oxysporum* by affecting the signaling of the target of rapamycin pathway (TOR). The TOR signaling system is essential in regulation of cell development in several eukaryotes. However, it is uncertain, whether this pathway plays any role in controlling the virulence of plant pathogenic fungi (Yu et al. 2014). According to Quiroz-Figueroa et al. (2001), exogenous administration of SA *in vitro* culture increased somatic embryogenesis in *Coffea arabica*.

Luo et al. (2001) found that SA stimulates organogenesis and embryogenesis by regulating cell division, enlargement, or activating DNA replication. SA stimulates the growth and development of shoots and roots of bean plants (Sanaa et al. 2001).

In our experiment, except for the high concentration of 2.5 mM, all concentrations of SA included in MS medium greatly enhanced explant development, number, and vegetative growth characteristics compared with the control. The inhibitory effects of high SA concentrations observed in previous investigations and the current study might be due to the toxic effects of SA that have been described in some plant species due to its stimulation of ROS biosynthesis (Singh & Usha 2003; Sanzani et al. 2014).

In our experiment, SA treatments (1.0, 1.5, and 2.0 mM) enhanced the length of shoots and roots and chlorophyll content. This beneficial effect of SA promotes plant development, directly or indirectly. According to a study by Alutbi et al. (2017), SA at a concentration of 100 $mg \cdot L^{-1}$ significantly increased the number of shoots and the length of shoots and roots in tissue culture of *Solanum tuberosum*. Jesus et al. (2015), studying the role of phytohormones in the response of *Eucalyptus globulus* plants to water deficit, used immunolocalization techniques to visualize changes in IAA and ABA levels after application of different SA concentrations (0.0, 0.75, 2.5, and 5.0 mM). The findings of their study provided valuable insight into the defense mechanisms of these plants. Multiple mechanisms are involved in the action of ABA as a negative regulator of signaling pathways that mediate plant defense. Increased desiccation resistance and inhibition of precocious germination promotes somatic embryogenesis and enhances the quality of somatic embryos *in vitro*.

Additionally, it has been proposed that auxin triggers a stage of plant development (Nic-Can & Loyola-Vargas 2016). Auxin gradient formation is essential for the initiation of somatic embryogenesis in *Eleutherococcus senticosus* and the induction of stem cell production in *Arabidopsis* embryonic calli (Choi et al. 2001; Su & Zhang 2009). All these findings emphasize the possibility of increasing the efficiency of somatic embryogenesis and enhancing plant tissue culture methods.

CONCLUSION

Salicylic acid at concentrations of 1–2 mM reduced the mycelial growth of fungi isolated from contaminated date palm cultures and lowered the percentage of contaminated cultures and necrotic calli. SA added to MS medium at concentrations of 1.0, 1.5, and 2.0 mM boosted the rate of embryogenic cultures, the number and length of shoots and roots, and the chlorophyll content. These factors increased ABA and IAA and decreased IBA concentration in leaves. The recommended SA concentration in *in vitro* culture of date palm is 1.5 mM.

Conflict of interest

The author declare no conflict of interest.

Ethical approval

This article does not contain any studies involving human or animal participants performed by any of the authors.

REFERENCES

Abass M.H. 2013. Microbial contaminants of date palm (*Phoenix dactylifera* L.) in Iraqi tissue culture laboratories. Emirates Journal of Food Agriculture 25(11): 875–882. DOI: 10.9755/ejfa.v25i11.15351.

Al-Mayahi A.M.W. 2016. Influence of salicylic acid (SA) and ascorbic acid (ASA) on *in vitro* propagation and salt tolerance of date palm (*Phoenix dactylifera* L.) cv. 'Nersy'. Australian Journal of Crop Science 10(7): 969–976. DOI: 10.21475/ajcs.2016.10.07.p7640.

Al-Mussawii M.A.Y. 2010. The source of bacterial contamination of date palm (*Phoenix dactylifera* L.) grown *invitro*. Basrah Journal for Date Palm Research 9(2): 132–146. [in Arabic with English abstract]

Alutbi S.D., Al-Saadi S.A.A.M., Madhi Z.J. 2017. The effect of salicylic acid on the growth and microtuberization of potato (*Solanum tuberosum* L.) cv. Arizona propagated *in vitro*. Journal of Biology, Agriculture and Healthcare 7(2): 64–70.

Amin A.A., Rashad El-Sh.M., Gharib F.A.E. 2008. Changes in morphological, physiological and reproductive characters of wheat plants as affected by foliar application with salicylic acid and ascorbic acid. Australian Journal of Basic and Applied Sciences 2(2): 252–261.

Barnes J.D., Balaguer L., Manrique E., Elvira S., Davison A.W. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. Environmental and Experimental Botany 32(2): 85–100. DOI: 10.1016/0098-8472(92)90034-y.

Barnett H.L., Hunter B.B. 1972. Illustrated Genera of Imperfect Fungi, 3rd ed. Burgess Publishing, USA, 241 p.

Chen L., Zhao X., Wu J., He Y., Yang H. 2020. Metabolic analysis of salicylic acid-induced chilling tolerance of banana using NMR. Food Research International 128; 108796; 10 p. DOI: 10.1016/j.foodres.2019.108796.

Choi Y.E., Katsumi M., Sano H. 2001. Triiodobenzoic acid, an auxin polar transport inhibitor, suppresses somatic embryo formation and postembryonic shoot/root development in *Eleutherococcus senticosus*. Plant Science 160(6): 1183–1190. DOI: 10.1016/s0168-9452(01)00357-0.

Cowan M.M. 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12(4): 564–582. DOI: 10.1128/cmr.12.4.564.

De Vleesschauwer D., Gheysen G., Höfte M. 2013. Hormone defense networking in rice: tales from a different world. Trends in Plant Science 18(10): 555–565. DOI: 10.1016/j.tplants.2013.07.002.

Ding Y., Sun T., Ao K., Peng Y., Zhang Y., Li X., Zhang Y. 2018. Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. Cell 173(6): 1454–1467. DOI: 10.1016/j.cell.2018.03.044.

Domsch K.H., Gams W., Anderson T.-H. 1993. Compendium of Soil Fungi, vol. 1. IHW-Verlag, Germany.

El-Taher A.M., Abd El-Raouf H.S., Osman N.A., Azoz S.N., Omar M.A. et al. 2022. Effect of salt stress and foliar application of salicylic acid on morphological, biochemical, anatomical, and productivity characteristics of cowpea (*Vigna unguiculata* L.) plants. Plants 11(1); 115; 15 p. DOI: 10.3390/plants11010115.

Guo B., Liu C., Liang Y., Li N., Fu Q. 2019. Salicylic acid signals plant defense against cadmium toxicity. International Journal of Molecular Sciences 20(12); 2960; 19 p. DOI: 10.3390/ijms20122960.

Jassim N.S., Ahmed A.N. 2024. The isolation and molecular identification of the main fungus caused leaf spots on date palms (*Phoenix dactylifera* L.). Archives of Phytopathology and Plant Protection 57(7): 542–554. DOI: 10.1080/03235408.2024.2375038.

Jassim N.S., Salih A.M., Ati M.A. 2021. Evaluating the efficiency of plants essential oils against common fungal contamination affecting tissue culture of date palms (*Phoenix dactylifera* L.) by *in vitro* culture. Research Journal of Chemistry and Environment 25(6): 40–45.

Jazi S.A., Yazdi H.L., Ranjbar M. 2011. Effect of salicylic acid on some plant growth parameters under lead stress in *Brassica napus* var. Okapi. Iranian Journal of Plant Physiology 1(3): 177–185.

Jesus C., Meijón M., Monteiro P., Correia B., Amaral J., Escandón M. et al. 2015. Salicylic acid application modulates physiological and hormonal changes in *Eucalyptus globulus* under water deficit. Environmental and Experimental Botany 118: 56–66. DOI: 10.1016/j.envexpbot.2015.06.004.

Kamboj J.S., Blake P.S., Quinlan J.D., Baker D.A. 1999. Identification and quantitation by GC-MS of zeatin and zeatin riboside in xylem sap from rootstock and

scion of grafted apple trees. Plant Growth Regulation 28(3): 199–205. DOI: 10.1023/a:1006292309765.

Karuppaiah P., Rameshkumar S., Shah K., Marimuthu R. 2003. Effect of antitranspirants on growth, photosynthetic rate and yield characters of brinjal. Indian Journal of Plant Physiology 8(2): 189–192.

Kelen M., Çubuk Demiralay E., Şen S., Özkan G. 2004. Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99 R (*Vitis berlandieri* × *Vitis rupestris*) and rose oil (*Rosa damascena* Mill.) by reversed phase liquid chromatography. Turkish Journal of Chemistry 28(5): 603–610.

Klessig D.F., Tian M., Choi H.W. 2016. Multiple targets of salicylic acid and its derivatives in plants and animals. Frontiers in Immunology 7; 206; 10 p. DOI: 10.3389/fimmu.2016.00206.

Leifert C., Cassells A.C. 2001. Microbial hazards in plant tissue and cell cultures. In vitro Cellular and Developmental Biology – Plant 37(2): 133–138. DOI: 10.1079/ivp2000129.

Luo J.-P., Jiang S.-T., Pan L.-J. 2001. Enhanced somatic embryogenesis by salicylic acid of *Astragalus adsurgens* Pill.: relationship with H_2O_2 production and H_2O_2-metabolizing enzyme activities. Plant Science 161(1): 125–132. DOI: 10.1016/s0168-9452(01)00401-0.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15(3): 473–497. DOI: 10.1111/j.1399-3054.1962.tb08052.x.

Nic-Can G.I., Loyola-Vargas V.M. 2016. The role of the auxins during somatic embryogenesis. In: Loyola-Vargas V.M., Ochoa-Alejo N. (Eds.), Somatic Embryogenesis: Fundamental Aspects and Applications. Springer, pp. 171–182. DOI: 10.1007/978-3-319-33705-0_10.

Qi P.-F., Johnston A., Balcerzak M., Rocheleau H., Harris L.J., Long X.-Y. et al. 2012. Effect of salicylic acid on *Fusarium graminearum*, the major causal agent of fusarium head blight in wheat. Fungal Biology 116(3): 413–426. DOI: 10.1016/j.funbio.2012.01.001.

Quiroz-Figueroa F., Méndez-Zeel M., Larqué-Saavedra A., Loyola-Vargas V.M. 2001. Picomolar concentrations of salicylates induce cellular growth and enhance somatic embryogenesis in *Coffea arabica* tissue culture. Plant Cell Reports 20(8): 679–684. DOI: 10.1007/s002990100386.

Radojičić A., Li X., Zhang Y. 2018. Salicylic acid: a double-edged sword for programed cell death in plants. Frontiers in Plant Science 9; 1133; 5 p. DOI: 10.3389/fpls.2018.01133.

da Rocha Neto A.C., Maraschin M., Di Piero R.M. 2015. Antifungal activity of salicylic acid against *Penicillium expansum* and its possible mechanisms of action. International Journal of Food and Microbiology 215: 64–70. DOI: 10.1016/j.ijfoodmicro.2015.08.018.

Sanaa Z.A.M., Ibrahim S.L., Sharaf E.H.A. 2001. The effect α-naphthalene acetic acid (NAA), salicylic acid (SA) and their combinations on growth, fruit setting, yield and some correlated components in dry bean (*Phaseolus vulgaris* L.). Annals of Agricultural Sciences 46(2): 451–463.

Santner A., Calderon-Villalobos L.I.A., Estelle M. 2009. Plant hormones are versatile chemical regulators of plant growth. Nature Chemical Biology 5(5): 301–307. DOI: 10.1038/nchembio.165.

Sanzani S.M., Schena L., Ippolito A. 2014. Effectiveness of phenolic compounds against citrus green mould. Molecules 19(8): 12500–12508. DOI: 10.3390/molecules190812500.

Singh B., Usha K. 2003. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regulation 39(2): 137–141. DOI: 10.1023/a:1022556103536.

Singh J., Tripathi N.N. 1999. Inhibition of storage fungi of blackgram (*Vigna mungo* L.) by some essential oils. Flavor and Fragrance Journal 14(1): 1–4. DOI: 10.1002/(sici)1099-1026(199901/02)14:1<1::aid-ffj735>3.0.co;2-r.

Su Y.H., Zhang X.S. 2009. Auxin gradients trigger de novo formation of stem cells during somatic

embryogenesis. Plant Signaling and Behavior 4(7): 574–576. DOI: 10.4161/psb.4.7.8730.

Suhaib M., Ahmad I., Munir M., Iqbal M.B., Abuzar M.K., Ali S. 2018. Salicylic acid induced physiological and ionic efficiency in wheat under salt stress. Pakistan Journal of Agricultural Research 31(1): 79–85. DOI: 10.17582/journal.pjar/2018/31.1.79.85.

Turkyilmaz B. 2012. Effects of salicylic and gibberellic acids on wheat (*Triticum aestivum* L.) under salinity stress. Bangladesh Journal of Botany 41(1): 29–34. DOI: 10.3329/bjb.v41i1.11079.

Wu H.-S., Raza W., Fan J.-Q., Sun Y.-G., Bao W., Liu D.-Y. et al. 2008. Antibiotic effect of exogenously applied salicylic acid on *in vitro* soilborne pathogen, *Fusarium oxysporum* f. sp. *niveum*. Chemosphere 74(1): 45–50. DOI: 10.1016/j.chemosphere.2008.09.027.

Yu F., Gu Q., Yun Y., Yin Y., Xu J.-R., Shim W.B., Ma Z. 2014. The TOR signaling pathway regulates vegetative development and virulence in *Fusarium graminearum*. New Phytologist 203(1): 219–232. DOI: 10.1111/nph.12776.

Zamani E., Sanjarian F., Mohammadi-Goltapeh E., Safaie N. 2019. Effects of salicylic acid on the growth and pathogenicity of *Zymoseptoria tritici*. Biological Journal of Microorganism 7(28): 53–62. DOI: 10.22108/bjm.2018.103408.1046.