Effects of methyl jasmonate and salicylic acid on the production of metabolites in *Portulaca oleracea* L. suspension culture

Fereshte Enaiate[1], Monireh Ranjbar[1]*, Sarvenaz Bigham Soostani[2]

1 Department of Biology, Flavarjan Branch, Islamic Azad University, Isfahan, Iran
2 Department of Biology, Faculty of Marian Science and Technology, Hormozgan University, Hormozgan, Iran.

Abstract

Purslane is a medicinal herb that is used as an antioxidant mostly because of its secondary metabolites. Inducing cell suspensions is a crucial biotechnological technique for altering the pattern of secondary metabolite production. The current study examined the impact of growth hormones, jasmonic acid (JA) and salicylic acid (SA), on the bioactive components of suspension-cultured *Portulaca oleracea* L. To induce callus, stem segments were cultivated on Murashige and Skoog (MS) medium supplemented with 6-Benzylaminopurine (BAP) and2,4-Dichlorophenoxyacetic acid (2,4-D). Cell suspensions were initiated from the callus, and metabolites were extracted after elicitation with JA and SA. In the treated groups, measurements were made of antioxidant activity, total phenols, flavonoids, saponins, alkaloids, and terpenoids. Concomitant use of JA and SA increased antioxidant capacity compared to individual treatments. The increase in total phenol and flavonoids was almost proportional to the increase in antioxidant capacity. The amount of saponin in combined JA treatments (250 µM) with both concentrations of SA was the same as in single SA treatments. Combination treatment of JA and SA at both concentrations had the same effect on alkaloid production. The highest amount of terpenoids was induced with SA treatment with a concentration of 50 µM. Simultaneous treatment of *P. oleracea* L. suspension with JA and SA increased antioxidant capacity by increasing the bioactive compounds of total phenol, alkaloids, and terpenoids.

Keywords: antioxidant capacity, alkaloids, jasmonic acid, purslane, salicylic acid, total phenols

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Introduction

Purslane (*Portulaca oleracea* L.) is an annual plant with fleshy stem and leaves. The plant grows in many parts of the world, including the Mediterranean region, Africa, and Asia (particularly the Middle East). It is native to both temperate and tropical climates. The reason for the importance of purslane in the food industry is the presence of polysaccharides, fatty acids, sterols, proteins, vitamins, and minerals. *P. oleracea* L. is a natural source of antioxidants, phenols, flavonoids, terpenoids, and alkaloids

* *Corresponding Author*
E-mail Address: : *Monireh.Ranjbar@iau.ac.ir*
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which is the reason for using it as a medicinal plant (Zhou et al., 2015).

Due to the low concentrations of bioactive compounds in purslane in its natural state, the plant can be stimulated using suitable techniques to produce more of these secondary metabolites (Fernandez-Poyatos et al., 2021). The use of suitable elicitors is one of the biotechnological strategies that works effectively for this purpose. Undifferentiated cells found in plant cell suspensions are utilized, under highly controlled circumstances, to increase the synthesis of secondary metabolites (Matsuura et al., 2017). Both biological and non-biological factors can impact the amount and modality of these secondary metabolites in agricultural products (Ncube et al., 2012). When exposed to elicitors, which cause the synthesis of secondary metabolites, plants respond to these stimuli differently. To achieve the highest possible growth and yield of cultured cells, various factors should be considered during the preparation of cell suspension cultures. These include the type and concentration of the elicitor, the length of time the cells were exposed to the elevator, the age of the cells, the cell line type, plant hormones, and the composition of the nutrients. (Wang et al., 2017). In cell suspension cultures, jasmonic acid (JA) and salicylic acid (SA) stimulate the synthesis of secondary metabolites, which regulates how plants react to abiotic and biological stresses (Gadzovska et al., 2013).

Numerous studies have indicated how elicitors affect the rise in secondary metabolites. For example, methyl jasmonate and JA have been used in conjunction with gibberellic acid (GA) in *Artemisia absinthium* cell suspension culture to increase the accumulation of artemisinin (Ali et al., 2015). Also, methyl jasmonate has been applied to *Thevetia peruviana* cell suspension culture to enhance peruvoside production (Arias et al., 2010). Rincón et al. (2016) found that utilizing 100 mM JA and 10 mM abscisic acid enhanced the production of phenolic compounds in the cultivated T. *peruviana* callus (Rincón et al., 2016). Also, treatment of *Achillea millefolium* with methyl jasmonate during the flowering stage has reduced the anthocyanin content and increased flavonoid contents compared to control plants (Ghanati et al., 2014). The growth of lavender explants was found to increase with JA and SA treatments, and low concentrations of elicitors were successful in raising the level of carotenoids and polyphenols in explants grown in culture media containing 1.5 mg/L^{-1} SA and 0.5 mg/L^{-1} JA (Miclea et al., 2020).

Considering the identification of different metabolites in purslane and the importance of increasing their production for medicinal uses, this study focused to investigate the effect of methyl jasmonate and SA on the production of metabolites in plant cell suspensions.

Materials and Methods

Explant origin

We purchased *portulaca oleracea* seeds from Pakan Bazar Isfahan Company in Iran. Following disinfection of the seeds in 70% (v.v^{-1}) ethanol (30 seconds) and 1.5% (v.v^{-1}) sodium hypochlorite (15 minutes), in that order, they were washed three times with distilled water. Then the seeds were cultured on full strength Murashige and Skoog (MS) medium consisting of 3% (w/v^{-1}) sucrose and 0.7% (w/v^{-1}) plant agar (Dogan, 2022). The cultivated seeds were maintained at 25±2 ℃ with 16 hours of light and 8 hours of darkness at a rate of 65 µmol/m^{-2}. Cool white fluorescent lights were used to provide light, and the germinated seeds were then used to create seedlings. Finally, under carefully monitored circumstances, seedlings were raised to provide explants (Sedaghati et al., 2018).

Callus induction

Stem pieces with lengths of 1 to 1.5 cm were cultured on complete MS (Dogan, 2022) for callus induction. The culture medium consisted of 3% (w/v^{-1}) sucrose, 0.7% (w/v^{-1}) agar, and different concentrations of combined 6-Benzylaminopurine (0.5–2.0 mg/L^{-1}) and 2,4-Dichlorophenoxyacetic acid (0.5–2.0 mg/L^{-1}). The pH of the medium was adjusted to 5.80± 0.02 prior to autoclaving. In each callus induction medium, ten explants were placed with six replications. All the cultures that were ready were kept at a temperature of 25±2 ℃ in the dark. About a week after culturing, callus

generation from explants was observed (Rodriguez-Sancheza et al., 2020).

Plant cell suspensions organization

After the callus was cultivated for 30 days, 2 g of the white, fragile callus was placed in a 100 ml Erlenmeyer flask containing 20 ml of autoclaved MS medium, enhanced with 0.5 mg/L^{-1} BAP ,100 mg/L Inositol, 30 g/L^{-1} sucrose, 1 mg/L^{-1} 2,4-D, and 1 mg/L thiamine (pH = 5.8). On day 15, 10 ml of the medium was taken out and replaced with 10 ml of fresh MS medium. The cell suspensions were continuously exposed to white light at 25±2 ℃ and agitated at a rate of 110 x g. (Rodriguez-Sancheza et al., 2020).

Cell suspensions elicitation

Ten (10) milliliters of the previously homogenized cell suspension were added to a 100 ml Erlenmeyer flask containing 10 ml MS medium. The initial concentration of each suspension was 90 g (FW/L^{-1}). After 15 days, JA and SA with the concentration of 25, 50, 250, and 500 µM, were added to the cell suspensions separately and in combination. In 1 ml of 0.25% ethanol, elicitor solutions were produced and filtered through a 0.22 µm membrane filter. The effects of solicitors were assessed 24 hours after they were added to the cell suspension. Ethanol 50% (v/v^{-1}) was utilized as a control in every experiment. A vacuum filter was used to separate the cells from the cell suspension media. Until the metabolites were removed, the cells were maintained at -80 ℃ (Rodriguez-Sancheza et al., 2020).

Extraction of intracellular metabolites

The biomass was dried in a convection oven at 60 ℃ for 48 hours. The obtained dry material was powdered. Using 15 ml of hydro ethanol 50 (v/v^{-1}), 0.3 g of powder was extracted in an ultrasonic bath (40 kHz) at a temperature of 30 ℃ for 30 min. Subsequently, the extracts were centrifuged for 10 minutes at 3000 × g. At last, the supernatant was collected and kept chilled at 4 ℃ (Rodriguez-Sancheza et al., 2020).

Determination of phenolic compounds

Using gallic acid as a standard, the Folin-Ciocalteu method was used to determine the amounts of phenolic compounds (Xu, 2022). The PCC in every sample was determined and recorded in mg GAE. g DW^{-1} (mg gallic acid equivalents per g DW).

Determination of flavonoid contents

The flavonoid-aluminum (AlCl3) complex method, which is based on the quercetin standard, was used to identify flavonoid compounds. The absorption at 425 nm was measured, and $AlCl_3$-free samples were used as blank. For calibration, alcoholic quercetin solutions containing 200, 100, 25, 12.5, and 6.25 mg/ml^{-1} were utilized. The data were presented as mg QE/g DW^{-1}, or milligrams of quercetin equivalent per gram dry biomass weight (Pekal and Pyrznska, 2014).

Determination of antioxidant activity (DPPH) radical scavenging

To achieve this, 2 ml of DPPH methanol dilution was mixed with 1 ml of methanolic extract from each of the samples. The mixture was maintained at room temperature in darkness, and a UV-30 spectrophotometer (SHIMADZU UV-1800) was used to measure the absorbance at a wavelength of 517 nm. DPPH was diluted with methanol to create the blank. Per mg dry weight, the results were expressed in mg equivalent to quercetin (Chaves et al., 2012).

Determination of alkaloid contents

A portion of the extract was filtered after dissolving in 2N HCL. After that, 1 ml of the solution was put in a separatory funnel and washed three times with 10 ml of chloroform. The pH of the resultant solution was adjusted using 0.1 N NaOH. After that, the solution was mixed with 5 ml of phosphate buffer and 5 ml of bromocresol green solution. After agitating the sample vigorously, 1, 2, 3, and 4 ml of chloroform solvent were used for the extraction, and then the same solvent was diluted. In a UV spectrophotometer (SHIMADZU UV-1800), the absorbance of the solution was measured at 470 nm in comparison with a blank not containing atropine (Ajanal et al., 2012).

Determination of saponins

The amount of total saponin was determined using the vanillin-sulfuric method. The absorbance of the sample was determined using a microplate reader (PowerWave XS-BT-MQX200R) at a wavelength of 544 nm. Milligrams of diosgenin per gram of dry extract were used to report the total saponin content (Le et al., 2018).

Determination of terpenoids

In 1 ml of methanol, 10 mg extract was dissolved. After that, 0.2 ml of every extract and the reference were combined individually with a mixture comprising 0.3 ml of 5% vanillin and 1 ml of perchloric acid. The obtained solutions were then heated at 60 ℃ for 45 min. The absorbance was measured at 548 nm after 5 ml of glacial acetic acid was added and allowed to cool. The reference was ursolic acid (Tukowski et al., 2022).

Determination of vitamin C

Plant samples were frozen in nitrogen liquid. Each sample was first ground to 50 mg, and then 2 ml of 5% (w/v^{-1}) TCA was added. After that, it was centrifuged for 10 minutes at 4 ℃ at 16000 ×g. Vitamin C was measured by incubating a reaction mixture at room temperature. Following incubation 50 µl TCA (10% w/v^{-1}), 50 µl phosphoric acid (44% w/v^{-1}), and 50 µl 4% (w/v^{-1}). After adding 2, 2-bipyridyl and 25 µl 3% (w/v^{-1}) FeCl3, the reaction mixture was ready to be incubated for 60 minutes at 37 ℃. The supernatant's absorbance was measured at 525 nm (Rbelo et al., 2020).

Determination of vitamin E

The samples were ground up and frozen in liquid nitrogen. The suspension was homogenized after 1 ml of hexane solvent was added for every gram of the sample. After centrifugation, the supernatant was re-dissolved in the solvent and then centrifuged again. Then, the supernatant and the extract were combined and stored in a refrigerator. Following that, 1 ml of the reagent was combined with 0.1 ml of hexane extract that was extracted from vitamin E-containing samples, and the mixture was incubated for 90 minutes at 37 ℃. Finally, at a wave length of 695 nm, the aqueous phase's absorption was measured (Samrot and Sean, 2022).

Statistical Analysis

To find out how elicitors affected the quantity of secondary metabolites, a factorial design experiment with a fully randomized design was employed. All experiments were performed in three replicates. Data analysis was done with SPSS software using ANOVA method. Means were compared using Duncan's test. Excel was used to draw the graphs.

Results

Effects of JA and SA on free radical scavenging, total phenols, and flavonoids content

Based on the results obtained by DPPH method (Table 1), the difference in free radical scavenging in treatments was significant (P<0.05). The highest percentage of inhibition (30% compared to the control) was seen in both JA and SA treatments. The increase in inhibition of free radicals was proportional to the increase in JA concentration, and SA alone was able to inhibit free radicals more than the control (Fig l. A).

Table 1
Results from the analysis of the variance of JA and SA on the data

Source of Variation	df	Free Radical Inhibition (%)	Total Phenols (mg/g DW)	Flavonoids (mg/g DW)	Saponin (mg/g DW)	Alkaloids (mg/g DW)	Terpenoids (mg/g DW)	Vitamin C (µM/g FW)	Vitamin E (µM/g FW)
JA	3	75.5*	324.1**	23.45*	0.47**	341.6**	263.3*	0.97**	13.56*
SA	3	81.31**	374**	46.76*	0.39*	523.8**	482.52**	0.74**	9.46^{ns}
JA*SA	6	72.1*	25931**	85.35**	072*	438.53**	168.37**	0.35*	46.28*
CV		0.59	0.52	0.46	0.17	0.095	0.31	0.032	0.013

ns: non-significant; *and **: significant at 5% and 1% levels of probability, respectively.

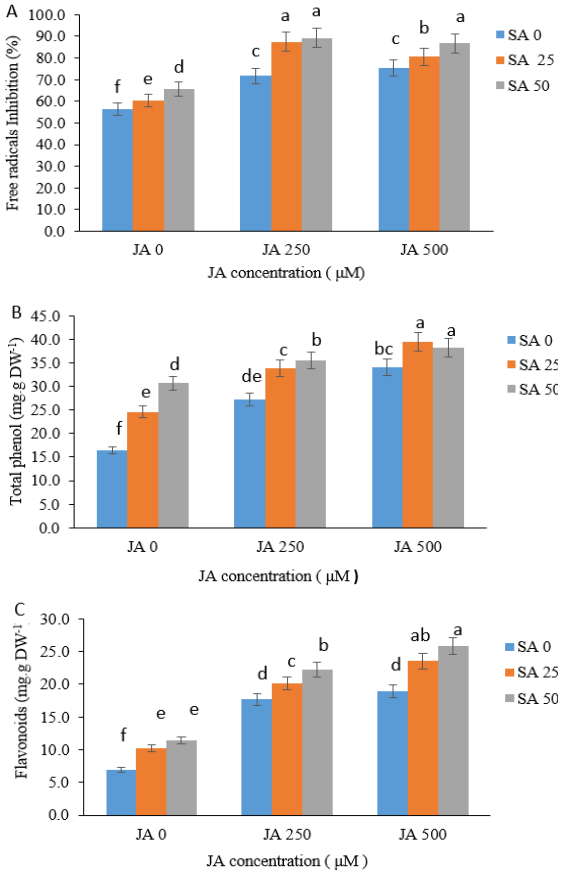

Fig. Ⅰ. Comparison of (A) free radical scavenging (B) total phenol content, and (C) flavonoid content in *Portulaca oleracea* L. following treatment with JA and SA; the data is an average of three replicates ± SD. different letters indicate significant differences among the treated groups.

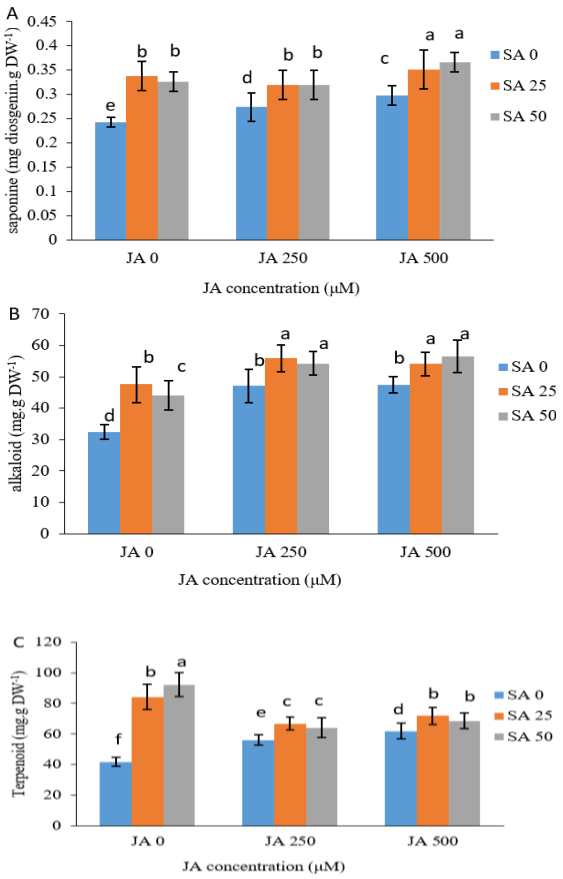

Fig. Ⅱ. Comparison of (A) saponin, (B) total alkaloid, and (C) total terpenoid contents in different treatments on *Portulaca oleracea* L.; data is an average of three replicates ± SD. Different letters indicate significant differences among the treated groups.

In the combined treatments, especially 500 µM JA with both SA concentrations, more total phenol was detected in the plants compared with the other groups. In these plants, total phenol was more than doubled compared to the control. In comparison to the control, basic treatments for JA and SA showed an increasing trend in total phenol. The total content of phenol was higher in the combined treatments as compared with simple JA treatment. The increasing trend of free radical scavenging was consistent with the amount of total phenol present. Therefore, these compounds were probably responsible for scavenging free radicals (Fig Ⅰ. B).

Changes in flavonoid levels in different treatment groups were largely similar to changes in the amount of total phenol in these groups (Fig Ⅰ. C). Furthermore, flavonoid contents in the combined groups ranged from 6.87 to 25 mg/g DW, which is 3–4 times more than those of the control. Also, the use of SA resulted in less flavonoid production than the control.

Changes in saponin levels, total alkaloids, and total terpenoid content

Table 1 shows that there were significant effects of both JA and SA, alone and in combination, on saponin levels. The control group had the lowest amount of saponin (0.23 mg/gDW). Groups that received co-treatment had more saponin than simple treatments. The amount of saponin increased from 0.27 and 0.29 mg/g dry weight in simple JA treatment to 0.31 and 0.36 mg/g DWt in combined treatments. SA had a greater effect on saponin levels than JA (Fig Ⅱ. A).

Table 1 demonstrates that there was a significant ($P<0.01$) impact of the JA and SA treatments on the overall alkaloid contents. Alkaloid contents rose in all treatments when compared to the control. Only in the combined treatment of 500 JA with 50 SA, did it increase from 31 mg/g DW to 56 mg/g DW). Combined treatments had more alkaloids than simple JA treatments (from about 45 mg/g DW in single JA treatment to 56 mg/g DW in combined treatments). However, increasing the JA concentration in the combined treatments had no effect on the alkaloid contents. In simple SA treatments, the effect of 25 µM was greater than that of 50 µM (Fig Ⅱ. B).

Compared to JA, SA had a bigger impact on the synthesis of terpenoids in cell culture. This amount increased from 41.77 mg/g in the control to 61.83 mg in the JA treatment and to 92.16 mg/g in the salicylic acid treatment. The maximum total terpenoid content was observed in the treatment with 50 µM SA. Combined treatments led to higher levels of terpenoids than simple treatments with JA at the same concentrations (Fig. Ⅱ. C).

Effects of JA and SA on vitamin C and vitamin E content

Although they are not considered secondary metabolites, vitamin C (ascorbic acid) and E (tocopherol) have antioxidant qualities, which is why they were found in the plants under study. The control group exhibited the highest level of these vitamins due to the lack of positive effects from the treatments (Table 1 and Fig. III. A, & B). The combined treatment of 250 µM JA with both concentrations of SA did not differ significantly in vitamin C contents compared to JA at the same concentration. Concentrations of 500 µM JA with both concentrations of SA were not different in terms of vitamin C content but were higher than JA at the same concentration (Fig. III. A). Treatment with SA had no effect on the level of vitamin E because it did not show any difference with the control (Table 1). The use of JA reduced the level of vitamin content. On the other hand, combined treatments could not increase the amount of vitamin E compared to the control (Fig. III. B).

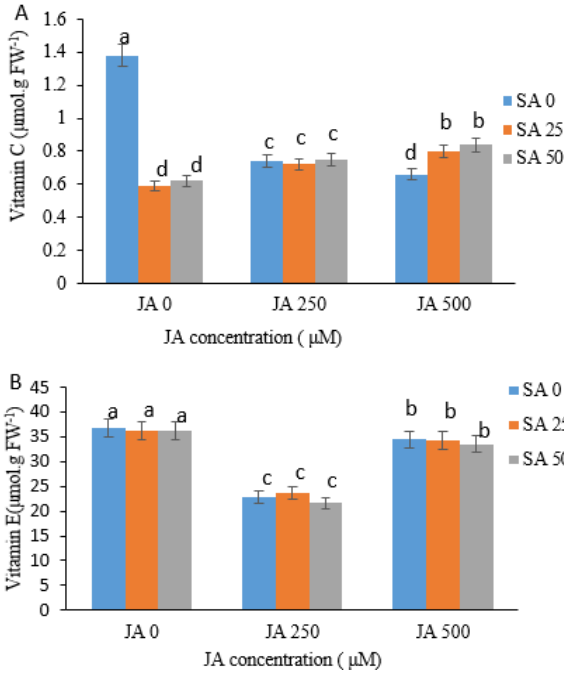

Fig. III. Comparison of (A) vitamin C (B) vitamin E contents in different treatments on *Portulaca oleracea* L. The data is an average of three replicates ± SD. Non similar letters indicate significant differences between different treated groups.

Discussion

Despite the fact that plants yield a wide range of therapeutic products, the complicated chemical makeup and unique spatial structure of these compounds make their industrial biosynthesis challenging. Nevertheless, given the effect of precursors and stimuli, plant cell culture is a good substitute for the ongoing generation of desirable secondary metabolites (Twaij and Hasan, 2022). Under laboratory circumstances, the process of producing secondary metabolites is divided into two stages. The first stage is mass manufacturing, while the second stage is secondary metabolite biosynthesis. These steps are independent of each other because each of them has different requirements and can be optimized independently (Scarepa et al., 2022). Elicitors are excellent tools for increasing the synthesis of secondary metabolites in biotechnological systems because they activate secondary biosynthetic pathways that govern carbon flow to obtain various beneficial plant chemicals. (Naik and Al-Khayri, 2016). The synthesis pathway of secondary metabolites involves a large number of control points that are regulated by facilitators.

This leads to the induction of transcription factors and the expression of particular genes. When a receptor and an elicitor interact, a signal transduction cascade is set off, which results in the execution of particular actions such as G protein activation, tyrosine kinase activation, calcium-mediated signaling, Janus kinase (JAK) pathway, phosphorylation, jasmonic-mediated early response genes, membrane acidification, DAG, IP3-mediated pathway, and cyclic adenosine-mediated pathway (Zhao et al., 2005). As a growth hormone, JA influences many different physiological and developmental reactions while also lowering the amount of expression of genes. The bioactive form of JA, jasmonoyl isoleucine (JA-Ile), is produced in the cytoplasm of tissues upon elicitation. (Li et al., 2017). Jasmonic acid-1 transfer protein (JAT1), which is found in the nuclear and plasma membranes

carries JA and JA-Ile from the cytoplasm to the nucleus and apoplast (Wang et al., 2019). Under normal conditions, transcription factors do not activate jasmonate-responsive genes. On the other hand, under certain conditions, JA specifically induces transcriptional and metabolic reprogramming in cells and tissues to promote the synthesis of secondary metabolites. (De Geyter et al., 2012). When methyl jasmonate and SA are used simultaneously in *Rehmannia glutinosa* Libosch, the result is a higher production of secondary metabolites than in the control (Piatezak et al., 2016). In *Portulaca oleracea* suspension cultures, treatment with JA or SA is a suitable alternative method that increases phytochemical production and cell growth. When cells treated with JA and SA produce high levels of phenolic compounds, flavonoids, and other bioactive compounds, it indicates that the compound has antioxidant potential (Fernandez-Poyatos et al., 2021). Numerous investigations have demonstrated that the stimulant methyl jasmonate increases the activity of a number of antioxidant enzymes (Ho et al., 2020). Probably, one of the reasons for increasing the inhibition of free radicals in the treatment with JA was due to the activity of antioxidant enzymes. Methyl jasmonate regulates catalase activity by increasing reactive oxygen species (ROS) production, thereby increasing the levels of secondary metabolites for large-scale production (Khan et al., 2019; Taj et al., 2019). These are in line with the findings of the present study.

Methyl jasmonate-treated adventitious roots in *Artemisia amygdalina* L. have demonstrated a high level of antioxidant activity, up to 89%. Also, a high antioxidant activity (87%) has been recorded in the methyl jasmonate-treated adventitious root suspension cultures of *Artemisia scoparia* (Taj et al., 2019). When JA is added to the culture medium, *Lavandula angustifolia* tissues develop strong antioxidant capacities (Andrys et al., 2018). SA and JA showed elevated antioxidant activity in elicited cells of *Panax ginseng* L. (Ali et al., 2007), *Artemisia absinthium* (Ali and Abbasi, 2014), and *Momordica dioica* (Chung et al., 2017) compared to the non-elicited cells. Treatment of *Hypercium perforatum* cell suspensions with JA increased phenyl ammoniacal lyase (PAL) and calcon isomerase (CHI) activity (Gadzovska et al., 2007).

One of the key enzymes in the phenylpropanoids biosynthesis chain, which is crucial for the synthesis of flavonoids, lignin, phenols, and many other related compounds, is PAL (Zhang et al., 2015). Treating cell cultures with methyl jasmonate was shown to increase the production of PAL enzymes, which has led to an increase in secondary metabolite production.

The phenolic content and taxol biosynthesis of cells also increased under SA treatment; therefore, increasing the concentration of SA induced the production of taxol (Caarls et al., 2015). In methyl jasmonate-treated cell suspension cultures with reduced anthocyanin content, the biosynthetic pathways from anthocyanins to phenolic compounds may have changed (AÇikgÖz et al., 2019). When JA was added to the *Lavandula angustifolia* Mill culture medium, the polyphenol content rose in comparison to the control (Andrys et al., 2018). In line with our findings, applying JA and SA raised the level of of total phenols and flavonoids in cell suspension cultures of *Panax ginseng* L. (Ali and Abbasi, 2014) and *Artemisia absinthium* L. (Ali et al., 2015). Optimal concentrations (100 and 200 µM) of methyl jasmonate and SA as well as optimal exposure period resulted in increased total

isoflavone in the cell suspension culture compared to the control (Halder et al., 2019). It has been recorded that the phyto-ecdysteroid levels increased following 14 days of treatment of *Ajuga bracteosa* with methyl jasmonate. Methyl jasmonate and phenylacetic acid (PAA) also increased the total phenolic and flavonoid contents in *A. bracteosa*. root suspension (Saeed et al., 2017). PAL activity has been stimulated by the use of JA and methyl jasmonate in the production of high levels of flavonoids (Park et al., 2019).

Panax ginseng cultures were also treated with 500 µM methyl jasmonate and produced 28-fold more saponin than the control (Lu et al*.,* 2001).A threefold increase in saponin production was observed when 0.2 mM SA was applied to adventitious roots of *Panax ginseng* L. (Lu et al*.,* 2001)JA increased the production of ginsenoside in cell suspension leading to an increase in total saponin content by synthesizing ginsenosides (Lu et al*.,* 2001). In *Glycyrrhiza glabra* var. violacea (Boiss.), treatment with 2 mM methyl jasmonate and 1 mM SA led to the increased saponin production by 3.8 and 4.5 times (Shabani et al., 2009). βAS, SS, and SE transcript levels in Medicago cell culture were unaffected by the inclusion of SA to the culture medium; however, 24 hours after the cultured cells were exposed to 500 µM methyl jasmonate, βAS transcription increased by about 50 times. (Suzuki et al., 2005).

In the 23-day-old hairy root culture of *Rehmannia glutinosa*, the addition of methyl jasmonate (50 µM) and SA (100 µM) in combination increased the production of iridoids (catalpol and harpagid), and phenylethanoids (verbascoside and isorbascoside) compared to the control (Piatezak et al., 2016). By treating hair root culture of *Rhinacanthus nasutus* with methyl jasmonate and SA, biomass accumulation decreased and the content of a group of naphthoquinone esters increased compared to the control (Cheruvathur and Thomas, 2014). When methyl jasmonate and/or SA are applied, the expression levels of important genes in the morphine biosynthesis pathway play an imperative part in the accumulation of these alkaloids at different times. Compared to the control, in methyl jasmonate treatment the expression of key genes coding Salsyn, SalR, SalAT, and CODM increased and caused the accumulation of thebaine, morphine, and codeine in plants. SA treatment increases morphine accumulation by increasing the regulation of Salsyn, T6ODM, and CODM genes (Halder et al., 2019).

The effect of different concentrations of methyl jasmonate (100, 150, and 200 mM) and SA (125, 250, and 500 mM) was investigated on dopamine production in *Portulaca oleracea* root culture. Results showed that treatment with 100 mM methyl jasmonate increased dopamine in cells by 4.3-fold compared to the control. Treatment with SA did not affect dopamine levels (Moghadam et al., 2001).

In *Tripterygium wilfordii* hair root culture, treatment with methyl jasmonate (50 µM) dramatically stimulated the production of a type of epitrophenidol (tryptolide) and wilfurine, although it has resulted in a minor reduction in the concentration of a sesquiterpene pyridine alkaloid. On the other hand, treatment with the same concentration of SA had no significant effect on hair root growth and had very little effect on the production of this secondary metabolite (Zhu et al., 2014).

SA influences post-translational modifications of transcription factors and regulators, which in turn influence the activity and localization of transcriptional regulators. Through thyrodoxin and glutardoxin, SA alters the transcriptional regulators responsible for the inhibition of JA-dependent genes. SA affects the transcription stimulated by JA. In order to activate JA signaling, JA-responsive transcription factors must first be destroyed and detached from their target genes. SA can then bind to suppressive proteins in the nucleus or bind to the genes in the cytosol. At the DNA level, changes in histone by SA-dependent factors suppress JA-dependent genes (Halder et al., 2019).

Conclusion

Concomitant treatment of *Portulaca oleracea* with JA and SA increased the antioxidant capacity of the cell suspension by increasing the levels of

secondary metabolites such as phenol, flavonoids, alkaloids, and terpenoids.

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