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Salicylic acid and methyl jasmonate enhance drought tolerance in chamomile plants

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ABSTRACT

Introduction: The dried flowers of chamomile contain many terpenoids and flavonoids contributing to its medicinal properties. Salicylic acid (SA) and methyl jasmonate (MeJA) have antioxidant properties and function as direct radical scavengers. Two *Matricaria chamomilla* cultivars (Bodgold and Hungary breed seeds) were used in this study to investigate the effects of exogenous application of SA and MeJA on protection against drought stress as well as on changes of malone dialdehyde (MDA) and electrolyte leakage index (ELI), and the fluctuation of proline and soluble sugars content in the leaves under drought stress.

Methods: The experiment was conducted in a factorial design based on randomized complete blocks with three replicates. Chamomile plants were treated by two levels of drought stress as well as two different levels of MeJA (i.e., 0.0 and 100 µM) and SA (i.e., 0.0 and 0.5 mM) solutions.

Results: There was a dramatic drought induced increase in the MDA content (128%) and ELI (49%) in the leaves. Deleterious effect of drought stress was more severe in untreated plants than in treated ones. Treatments with SA and MeJA significantly improved drought tolerance in chamomile plants. These treatments effectively maintained membrane integrity, thereby retarding electrolyte leakage and membrane lipid peroxidation (MDA). Treatments with SA and MeJA were also effective in enhancing the antioxidant concentrations of proline and soluble sugars.

Conclusion: The production of these antioxidants could have been part of a defence system against drought damage, reducing MDA and ELI and maintaining membrane stability.

Implication for health policy/practice/research/medical education:
The results indicate that SA and MeJA were found to individually enhance compatible solutes production in leaves but when administered in combination there was no further enhancement in compatible solutes production.

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Introduction

Chamomile (*Matricaria chamomilla* L.) is one of the important medicinal plant species from the Asteraceae family and represented by two common varieties viz. German Chamomile (*Chamomilla recutita*) and Roman Chamomile (*Chamaemelum nobile*). The flowers of chamomile contain terpenes, flavonoids, coline, coumarin, malic acid, proteins, sugars, lipids and mineral elements. About 120 chemical compounds have been identified in the oil, including 28 terpenes (the more important being α-bisabolol, chamazulene, bisabolol oxide, etc.), 36 flavonoids (apigenin, etc.) and other 52 substances that are organic acids, coumarins, coline, etc. (1).

Drought stress is often related to the production of oxygen radicals that play an important role in cell signaling, but at the same time is cytotoxic and need to be detoxified (2). Reactive oxygen species (ROS) acts as signaling molecules that triggers the cascade of protective reactions in plants (3), including activation of antioxidant enzymes and production of compatible solutes. Plant cells contain a repertoire of ROS scavenging enzymes and lipid-related substances like carotenoids (4), the putative compatible

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osmolytes including sorbitol, mannitol, myo-inositol and proline (5). A stress response is initiated when plants recognize stress at the cellular level. Plants produce jasmonic acid (and its volatile methyl ester methyl jasmonate (MeJA) and salicylic acid (SA) in response to many biotic and abiotic stresses.

In plants, SA plays an important role in signaling both local and systemic defense responses. SA is an endogenous growth regulator of phenolic nature, which participates in the regulation of different physiological processes in plants. SA has been recognized as a regulatory signal mediating plant response to abiotic stresses such as drought (6) and osmotic stress (7). Also, SA action is tightly related to the generation of various ROS (8).

The role of jasmonic acid (JA) and its volatile MeJA as signalling molecules in biotic and abiotic stresses (9) is well known, having either inhibitory or promoting effects, in the different form of morphological, physiological changes or in defence metabolism (10). In summary, it is clear that JA signaling exert its functions via interaction with multiple plant hormones; however the crossroads of these interactions still remain to be explored. We need the information for understanding the mechanisms of the anti-stress effects of SA and MeJA in medicinal plants under drought stress which are prerequisite to justify the use of these natural growth regulators for increasing *Matricaria Chamomilla* resistance to drought.

It was hypothesized that treatments with MeJA and SA may enhance drought tolerance in chamomile plants by enhancing the synthesis of biochemical compounds such as proline, ascorbic acid and soluble sugars. These biochemical compounds are thought to be involved in the response of chamomile plants to drought conditions and the signaling cascade are mediated with MeJA and SA. This study is aimed to answer whether MeJA and SA can function as stress molecules and how MeJA and SA mediate a stress response to diverse physiological and physical stresses.

Materials and Methods

Current investigation was conducted under greenhouse condition at the University of Tehran, Iran, from April to August 2013, to investigate the effects of MeJA, SA and different irrigation regimes on the synthesis of biochemical compounds (proline and soluble sugars content), cell membrane stability, determination of lipid peroxidation i.e., malone dialdehyde (MDA) in the leaves of two chamomile cultivars (Bodgold and Hungary breed seed). The seeds of two chamomile cultivars were kindly provided by Pakanbazr Institute, Isfahan, Iran. Subsequent to initial germination of the seeds in the pots supplemented with loamy soil and sand (3:1), the 30-day old seedlings were transferred into the new plastic pots (with the ratio of 3 loamy soil and 1 perlite) containing six ones. In the following, two out of which were thinned in providing suitable feeding conditions.

For the two months, pots were weighed every other day, and all plants were watered to field capacity (FC), supplying an amount of water equal to transpiration losses; to ensure the establishment of seedlings and to allow adaptation to surround conditions before water stress was imposed. By the end of this period, watering was discontinued.

Plants were watered to field capacity for about two months until roots were established and shoot system was in a reasonable size.

Experimental treatments applied as a combination of visual symptom appearance in plant (normal to severe wilting) and soil moisture. So, treatments irrigated in 20.1 and 10.4% of soil moisture content (w/w) that are created in greenhouse situation with irrigation of 4 and 10 days interval, respectively. Also chamomile plants were treated with the two different levels of MeJA (i.e., 0.0 and 100 μM) and SA (i.e., 0.0 and 0.5 mM) solutions. The first MeJA and SA treatments were applied after two months from transplanting and the process was repeated every other 10 days.

For proline determination, the last fully expanded leaves were detached from the plants after the drought treatment. Leaf samples (0.5 g) were homogenized in 5 mL of sulfosalicylic acid (3%) using mortar and pestle. The mixture was centrifuged at 9000 g for 10 min at 4 ˚C and the supernatant was recovered. About 2 mL of extract was taken in test tube and 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent were added to it. The reaction mixture was boiled in water bath at 100 ˚C for 60 min. The reaction was stopped by placing the test tubes in cold water. After cooling the reaction mixture, 6 mL of toluene was added and then transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and absorbance read at 520 nm in spectrophotometer against toluene blank. Concentration of proline was estimated by referring to a standard curve of proline (11).

To measure the content of soluble sugars, 0.5 g of dry leaves was homogenized with 5 ml of 95% ethanol. One-tenth ml of alcoholic extract preserved in refrigerator mixed with 3 ml anthrone (150 mg anthrone, 100 ml of 72% sulphuric acid, W/W). The samples placed in boiling water bath for 10 min. The light absorption of the samples was estimated at 625 nm using a PD-303 model spectrophotometer. Contents of soluble sugar were determined using glucose standard and expressed as mg g^{-1} DW of leaves.

Oxidative damage to leaf lipids, resulting from drought stress, was measured in terms of MDA content using thiobarbituric acid (TBA)-reactive substances (12). Leaf samples of 0.5 g were homogenized in 10 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 g for 5 min. Four milliliter of 0.5% TBA in 20% TCA was added to 2 mL of aliquot of the supernatant. The mixture was heated at 100 ˚C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of supernatant was

recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The following formula was applied to calculate malondialdehyde content using its absorption coefficient (ε) and expressed as nmol malondialdehyde g^{-1} fresh mass following the formula: MDA (nmol g^{-1} FM) = [(A532-A600) ×V×1000/ε] ×W where ε is the specific extinction coefficient (=155 mM cm^{-1}), V is the volume of crushing medium, W is the fresh weight of leaf, A600 is the absorbance at 600 nm wavelength and A532 is the absorbance at 532 nm wavelength.

In the middle of flowering stage cell membrane stability was analyzed. Leaf tissue (three plants/ replicate) was cut in segments of 1 cm^2 with cork-borer. Samples collected were washed three times in deionized water to remove electrolytes adhered on the surface (13). The samples were then kept in a capped vial (20 ml) containing 10 ml of deionized water and incubated in the dark for 24 h at 10±1 °C. The conductance was measured with a conductivity meter. After the first measurement the vials were autoclaved for 15 min to kill the leaf tissue and release the electrolytes. After cooling, the second conductivity reading was taken. These two measurements were carried out individually for all the samples from both the control and stress treatments. ELI was calculated as the reciprocal of cell membrane stability (CMS) (13): ELI% =1-[1-(T2-T1))/ (1-(C2-C1))]´100, where T and C refer to the stress and control samples, respectively; the subscripts 1 and 2 refer to the initial and final conductance readings, respectively.

The experiment was conducted in a factorial design based on randomized complete blocks with three replicates. Graphs were drawn using Microsoft Office Excel 2013 software.

Results

Data analysis showed that the factors including irrigation regimes, SA and MeJA (except MDA and soluble sugars) appear to influence the activity of proline, soluble sugar content, MDA and ELI. Also, interaction effect among three factors is significant at 1% level (Table 1). As in Table 1 is shown, interaction effect between SA and MeJA exhibited significant effects on all the aforementioned measured variables (P<0.01). In addition, a significant association was observed among irrigation regimes, SA and MeJA in terms of proline content (P<0.01), and ELI (P<0.05).

The free proline and soluble sugar contents were significantly enhanced in the stressed plants over control plants in both cultivars (Bodgold and Hungary breed seed). There was a considerable increase in free proline accumulation (19.72%) and soluble sugars (17.69%) with increasing drought stress. The results showed that the highest level of proline (24.05 n mol g^{-1} FW) and sugar (34 mg. g^{-1} DW) contents were achieved by applying SA under the condition of restrictive irrigation regime (I2) in Hungary breed seed, whereas the lowest amounts were related to the treatment of normal irrigation (I1) without using SA and MeJA (Table 2).

Table 1. Variance analysis for proline, soluble sugars content, MDA and ELI as effected by different irrigation regimes, SA and MeJA

S. O. V.	df	MS			
		Pro	MDA	ELI	Soluble sugar
Rep	2	2.1^{ns}	267.53^{**}	2.39^{ns}	85.93^{**}
Irrigation regimes (I)	1	71.72^{**}	302.65^{**}	1002.37^{**}	95.34^{**}
MeJA	1	7.55^{**}	8.34^{ns}	9.45^{**}	0.14^{ns}
SA	1	105.44^{**}	19.21^{*}	58.83^{**}	270.55^{**}
Cultivar	1	3.65^{*}	29.5^{ns}	161.5^{**}	0.69^{ns}
I * MeJA	1	24.97^{**}	4.47^{ns}	12.42^{**}	66.67^{**}
I * SA	1	10.64^{**}	5.5^{ns}	7.53^{*}	36.29^{*}
I * C	1	0.67^{ns}	0.03^{ns}	0.03^{ns}	1.93^{ns}
MeJA * SA	1	30.38^{**}	62.76^{**}	74.17^{**}	22.4^{*}
MeJA * C	1	4.84^{*}	5.98^{ns}	9.43^{**}	1.66^{ns}
SA * C	1	25.95^{**}	0.13^{ns}	0.54^{ns}	64.04^{**}
I * MeJA * SA	1	45.75^{**}	53.9^{**}	43.27^{**}	113.98^{**}
I * MeJA * C	1	1.45^{ns}	2.95^{ns}	2.81^{ns}	1^{ns}
I * SA * C	1	8.64^{**}	1.75^{ns}	6.06^{*}	0.22^{ns}
MeJA * SA * C	1	4.56^{*}	5.16^{ns}	0.05^{ns}	6.81^{ns}
I * MeJA * SA * C	1	0.83^{ns}	4.45^{ns}	0.16^{ns}	2.55^{ns}
Error	30	0.89	7.55	1.22	4.95
CV%		15	8	15	14

Pro: Proline content, MDA: Malone dialdehyde, ELI: Electrolyte leakage index, SA: salicylic acid, MeJA: methyl jasmonate, C: cultivar, df: degree of freedom; MS: mean of square; *,** significant at 0.05 and 0.01 probability levels, respectively. ns; non-significant.

Table 2. Mean proline content (µmol/g^{-1} F. W), soluble sugars (mg/g D.W), malondialdehyde (nmol g^{-1} F.W) and electrolyte leakage index (%) in chamomile under different irrigation regimes with or without SA and MeJA applications.

Irrigation regimes	Methyl jasmonate	Salicylic acid	Cultivar	Pro	Soluble sugars	MDA	% ELI
Normal	Control	Control	Bodgold	15.1 ± 0.93	23.06 ± 1.2	5.53 ± 1.52	23.71± 0.25
			Hungary breed seed	14.1 ± 0.12	20.40 ± 1.2	7.68 ± 2.8	28.71 ± .031
		0.5 mM	Bodgold	17.15 ± 0.35	25.1 ± 1.2	5.1 ± 1.42	22.68 ± 0.31
			Hungary breed seed	19.15 ± 0.35	27.91 ± 1.2	6.61 ± 2.55	25.73 ± 0.24
	100 µM	Control	Bodgold	15.81 ± 0.35	23.22 ± 1.2	5.4 ± 1.45	23.71 ± 0.31
			Hungary breed seed	17.15 ± 0.35	21.31 ± 3.6	7.04 ± 2.6	27.80 ± 0.29
		0.5 mM	Bodgold	20.34 ± 1.0	29.27 ± 2.23	5.2 ± 1.51	23.75 ± 0.25
			Hungary breed seed	21.15 ± 0.35	31.67 ± 3.03	6.39 ± 2.51	26.09 ± 0.24
Severe wilting	Control	Control	Bodgold	19.15 ± 0.35	27.91 ± 1.23	13.29 ± 4.74	36.85 ± 0.17
			Hungary breed seed	15.81 ± 0.35	23.22 ± 1.23	16.78 ± 6	41.26 ± 0.28
		0.5 mM	Bodgold	22.04 ± 0.35	31.98 ± 1.74	7.72 ± 2.58	28.78 ± 0.28
			Hungary breed seed	24.05 ± 0.35	34.07 ± 0.76	9.66 ± 2.95	34.55 ± 0.32
	100 µM	Control	Bodgold	20.71± 0.35	28.02 ± 2.23	9.911 ± 3.16	31.75 ± 0.28
			Hungary breed seed	20.04 ± 0.35	27.08 ± 2.23	8.46 ± 2.13	33.78 ± 0.25
		0.5 mM	Bodgold	17.22 ± 0.35	25.62 ± 0.74	10.63 ± 1.34	32.82 ± 0.57
			Hungary breed seed	20.49 ± 0.35	26.6 ± 2.03	12.68 ± 1.08	35.48 ± 0.45

Pro: Proline content, MDA: Malone dialdehyde, ELI: Electrolyte leakage index, Means followed by different lower-case letters are significantly different at (P< 0.05) by the Duncan's Multiple Range Test.

The treatment of seedlings with SA caused a significant increase in the concentrations of proline and soluble sugars in seedlings, which induced 24.3% and 34% growth in terms of proline content and 22% and 29.26% in terms of sugars under normal (I1) and severe drought stress (I2), respectively, compared with the control (Table 2).

Our results showed that lipid peroxidation (MDA) and electrolyte leakage index (ELI) were influenced by drought stress in leaves of chamomile. There was a dramatic drought induced increase in the MDA content (128%) and ELI (49%) in the leaves. The highest MDA content (16.78 n mol g^{-1} FW) and ELI (41.26%) were observed under the condition of restrictive irrigation regime (I2) in Hungary breed seed (without SA and MeJA treatments) (Table 2). The treatment of chamomile plants with SA caused a significant reduction in the content of MDA and ELI in seedlings, which induced 12.45% and 72.95% decrease in terms of MDA content and 8.3% and 23.34% in terms of ELI under normal (I1) and severe drought stress (I2), respectively, compared with the control. Pretreatment with MeJA brought about a 6.11% decrease in the ELI under water stress condition (Table 2).

Discussion

Plants have to cope with various environmental stresses and thus protect themselves using different strategies such as chemical defences. In general, concentrations of proline and soluble sugars of leaves increased with the decline in irrigation water, suggesting that the production of these solutes is probably a common response of chamomile under drought conditions. Non-enzymatic antioxidant system (low molecular metabolites) plays an important role in the process of neutralization of the after effects of oxidative stress.

The role of proline in adaptation and survival of plants under drought stress have been reported by many researchers (14,15). The accumulation of sugars in response to drought is also quite well documented (15,16). Proline accumulation may contribute to osmotic adjustment at the cellular level in many plants, suggesting it as one of the possible means for overcoming osmotic stress caused by the loss of water (17).

An imbalance between ROS production and antioxidant concentration (which resulted from stress condition) in a cell may cause damage to membranes leading to the

increase in membrane permeability. However, ROS are not only produced as a toxic by-product but also play a role in plant response to stress. These responses to stress include hormone signalling, gene activation and stomatal regulation (18). Enhanced ROS production can act positively to signal or induce protective mechanisms, such as accumulation of proline, soluble sugars, tocopherols and phenolic compounds. Induction of defences is mediated by phytohormones like JA and SA.

Because of low cost and good effects on secondary metabolites accumulation, MeJA and SA are considered as effective chemicals for large-scale cultures. Our study showed that SA and MeJA treatments increased proline and sugars concentrations whether plants were under stress or not. However, the most effects of mentioned hormones on compatible metabolites were more pronounced in stressed than that in non-stressed plants. Increasing the amount of proline and sugars in the plants would lead to the resistance against loosing water, protect turgor, reduce the membrane damage and accelerate the growth of plants in stress conditions (19).

Clearly, SA induced an increase in Abscisic acid (ABA) content that might be contributed to preadaptation of plants to different stressful influences of abiotic nature (20).

Exogenous application of methyl jasmonate is transported through phloem and xylem pathways (21). Through cell membranes MeJA is probably transported by the same or a similar carrier as sucrose, due to enhancement of the energy of the plasma membrane (21). Exogenously applied JA induced anthocyanin accumulation in different plants (22,23). Adding jasmonic acid to culture medium of sour cherry stimulated cyanidin 3-glucoside synthesis (24).

Our data show that SA and MeJA are involved in the regulation of proline and sugars production, these solutes may function as typical osmoprotectants, stabilizing cellular membranes and maintaining turgor pressure. Therefore, it is safe to suggest that these osmoprotectants contribute to the preadaptation effect of SA and MeJA on chamomile plants.

Plants drought tolerance was assessed by MDA content and ELI in tissues damaged by drought treatments. Estimation of MDA amount, which is a secondary end product of polyunsaturated fatty acid oxidation, is widely used to measure the extent of lipid peroxidation as indicator of oxidative stress (25). The lower membrane stability index reflects the extent of lipid peroxidation, which in turn is a consequence of higher oxidative stress due to water stress conditions.

Drought stress is well-known to cause a shift in the balance of plant cells. This shift is due to an increase in the rate of generation of ROS, which induce lipid peroxidation (LPO) in the membrane structures of the cells (26).

The treatment with SA and MeJA dramatically decreased MDA content and ELI in the leaves. This could be the consequence of the preadaptation effect of SA and MeJA on chamomile plants, in which hormone-induced events leading to activation of the low molecular metabolites were initiated before the exposure of the seedlings to severe drought stress. This may facilitate the strengthening of cell walls and effectively neutralize excessive (damaging) increase in the level of ROS during further exposure to the stress-factor, thereby preventing the damage of cellular membrane structures and changes in their permeability under stress conditions (27).

Finally, SA and MeJA were found to individually enhance compatible solutes production in leaves (especially SA), but when administered in combination there was no further enhancement in compatible solutes production. The combination treatment was also found to result in high MDA and ELI. The highest levels of compatible solutes were produced in the treatment with SA applied alone. The JA signal acts co-operatively with other plant hormones. The cross-talk between JA and SA can be synergistically or antagonistically, depending on particular stress (28).

Conclusion

Drought stress caused dramatic increase in MDA content and ELI in both cultivars. Deleterious effects of drought stress were more severe in untreated plants than in treated plants. Treatments with 100 µM MeJA and 0.5 mM SA enhanced seedling total antioxidant capacity. The enhancement of antioxidant capacity in chamomile plants could have contributed to the drought tolerance. This treatment effectively maintained membrane integrity, thereby retarding electrolyte leakage and membrane lipid peroxidation (MDA).

Authors' contributions

All the authors wrote the manuscript equally.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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