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Proceeding Paper

Effect of Salicylic Acid and Methyl Jasmonate on Stress Indices in *Papaver bracteatum* Lindl †

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Abstract: The Persian poppy (*Papaver bracteatum* Lindl.) is a perennial medicinal plant belonging to the Papaveraceae family endemic to the mountainous areas of Northern Iran. It is known for high amounts of the valuable benzylisoquinoline alkaloid thebaine. In this study, the effects of salicylic acid and methyl jasmonate elicitors on stress indices were investigated. For this purpose, three concentrations of salicylic acid and methyl jasmonate were applied in three different populations of Persian poppies. The interactions of population×salicylic acid×methyl jasmonate were significant (level of 1%) for chlorophyll fluorescence, ion leakage, malondialdehyde, and proline indices. The highest Fv/Fm (0.838) was observed in the German population with 100 µM salicylic acid treatment. The lowest ion leakage (20.51%) was observed in the Polour region population with 100 µM methyl jasmonate treatment. The lowest amount of malondialdehyde (19.36 µmol/g fresh weight) was observed in the Fil Zamin region population with 100 µM salicylic acid treatment. The highest amount of proline (6.29 µmol/g fresh weight) was also observed in the Polour population with 100 µM salicylic acid treatment. In general, salicylic acid and methyl jasmonate treatments were shown to improve stress-related indices. It seems that the best treatments by means of which to increase plant capacity to deal with environmental stresses are 100 µM salicylic acid and 100 µM methyl jasmonate in the Persian poppy.

Keywords: medicinal plants; chlorophyll fluorescence; ion leakage; malondialdehyde; proline

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1. Introduction

Persian poppy, with the scientific name *Papaver bracteatum*, is one of the three species belonging to the Oxytona section of the Papaveraceae family. All species in Oxytona are perennials and are propagated by seeds, usually producing flowering stems in the spring. Proper rosette growth before winter produces abundant flowers and fruits in the spring [1]. Known as the major secondary metabolite in roots, leaves, and capsules due to its high accumulation, thebaine is produced through the biosynthesis pathway of benzylisoquinoline. Other important drug alkaloids produced through this pathway include morphine, codeine, narcotine, oripavine, papaverine, and noscapine, among others [2]. Due to the non-narcotic and non-addictive nature of thebaine and the ease of its artificial conversion to other high-demand drugs, there is a growing international demand for thebaine-containing plants [3].

In 2021, almost 225 tons of natural compounds containing thebaine were sold globally; Australia, France, Hungary, Spain, and India had the largest shares in production [4].

Salicylic acid plays a vital role in regulating plant growth, development, the interaction between plant organs, and response to environmental stresses. In addition, its role in seed germination, fruit yield, glycolysis, heat generation during flowering, ion uptake and transfer, and photosynthesis have been revealed [5]. The use of methyl jasmonate in in vitro cultures has been shown to activate antioxidant enzymes and stimulate the expression of defense-related genes and the production of secondary metabolites [6]. It has been shown that environmental stresses, such as foliar application of salicylic acid and methyl jasmonate, can improve the production of secondary metabolites [7].

This study aimed to investigate changes in chlorophyll fluorescence, ion leakage, malondialdehyde, and proline under the influence of salicylic acid and methyl jasmonate treatments.

2. Materials and Methods

In this experiment, seeds of two different populations of Persian poppy were obtained kindly from the IPK company, Germany, and another single population was collected from the Polour region located on the south side of Mount Damavand in September 2019 using botanical information (*P. bracteatum* species have a straight stem without branching with a length of 50–80 cm and a single terminal flower, three to eight brackets (its scientific name is due to its brackets), four to six bold red petals with one or two black spots in the base, and long, elongated capsules with short, dense hairs, which can be identified on the sepals). For ease of reviewing the results, the population collected from the Polour region was named the first population; the next population, with the accession number PAP 832, of Iranian origin, from the Fil Zamin region, was named the second population; and, finally, the last population, with the accession number PAP 754, of German origin, was named the third population.

The present experiment was based on a factorial experiment in the frame of a randomized complete block design. Three levels of salicylic acid (control, 100, and 200 μM) and three jasmonic acid levels (control, 100, and 200 μM) were applied at four time points with 30-day intervals. Data analysis of the biochemical properties of three different populations of Iranian poppy and Duncan's Multiple Range Test were performed using SPSS Statistics 26 (IBM) software, purchased from the DigiKala online store, Tehran, Iran.

2.1. Chlorophyll Fluorescence

All chlorophyll fluorescence parameters (F_0, F_m, F_v/F_m) were measured with a portable chlorophyll fluorescence meter (handyPEA, hansatech Instruments, Pentney, UK).

2.2. Ion Leakage

Ion leakage was measured based on Sullivan and Ross [8].

2.3. Malondialdehyde

Malondialdehyde concentration was measured using the thiobaric acid method described by Ali et al. [9].

2.4. Proline

Free proline was measured according to Bates et al. [10]. The absorbance was read at 520 nm spectrophotometrically.

3. Results and Discussion

The analysis of variance results showed the maximum quantum efficiency of photosystem II, ion leakage, malondialdehyde, and proline with different concentrations of salicylic acid and methyl jasmonate, and their interaction was significantly different in all populations (level 1%) (Table 1).

Table 1. Results of the variance analysis of stress-related indices among the control and the salicylic acid and methyl jasmonate-treated Persian poppy populations.

SOV	df	Fv/Fm	Ion Leakage	MDA	Proline
Block	2	4.93×10^{-6}	0.571	0.151	0.051
Population	2	0.011 **	14.238 **	97.384 **	0.952 **
Salicylic Acid	2	0.017 **	196.221 **	190.825 **	8.180 **
Methyl jasmonate	2	0.008 **	81.799 **	139.810 **	16.507 **
Pop * Sa	4	0.010 **	9.842 **	0.508	2.948 **
Pop * MJ	4	0.009 **	6.363 **	1.427 **	3.710 **
Sa * MJ	4	0.018 **	362.301 **	280.765 **	10.931 **
Pop * Sa * MJ	8	0.011 **	5.657 **	2.268 **	1.184 **
E	52	2.43×10^{-6}	0.227	0.268	0.050
CV (%)		5.04	17.99	20.35	19.24

** 1% significance ($p < 0.01$); * 5% significance ($0.01 < p < 0.05$).

3.1. Chlorophyll Fluorescence

The highest quantum efficiency of photosystem II was observed in the Polour, Fil Zamin, and German populations, with values of 0.835, 0.832, and 0.838, respectively, in the treatment with 100 μM salicylic acid, which was, respectively, 14.86, 16.53, and 26.40% higher than the control treatment. The lowest quantum efficiency of photosystem II was obtained with 0.663, 0.714, and 0.727 in the control treatments of the German, Polour, and Fil Zamin populations, respectively.

It has been shown that the treatment of broccoli with nanomolar methyl jasmonate caused an increase of 61.8% in the quantum efficiency of photosystem II compared to the control treatment [11]. Khoshbakht and Asghari [12] showed that the quantum efficiency of photosystem II (Fv/Fm) in orange trees under salinity stress treatment decreased, but with salicylic acid treatment, its amount increased significantly. These results indicate the very favorable effect of salicylic acid and methyl jasmonate on chlorophyll fluorescence, which has improved photosystem II and increased photosynthetic efficiency.

3.2. Ion Leakage Percentage

The highest rate of ion leakage was observed in the Polour, Fil Zamin, and German populations, with values of 41.88, 40.65, and 39.80, respectively, in the control treatment. The lowest ion leakage rates of 20.51, 23.61, and 12.24 were obtained with 100 μM methyl jasmonate, 100 μM methyl jasmonate, and 200 μM salicylic acid treatments in the second, third, and first populations, respectively. These values are 42.41, 49.54, and 40.68% lower than those for the respective control treatments, which shows that 100 μM methyl jasmonate treatment in the second and third populations and 200 μM salicylic acid treatment significantly reduced the ion leakage of leaves.

It has been shown that under stressful conditions, oxidation and alterations of the structure/nature of protein and lipid components of membranes, by destroying their integrity, increase the leakage of electrolytes into the apoplastic space [13]. A study on Lemon Beebrush under salinity stress showed that foliar treatment of salicylic acid with a concentration of 1 mM significantly reduced ion leakage compared to the control [14]. Treatment of soybean plants with 500 μM methyl jasmonate has been shown to reduce the percentage of ion leakage of leaf samples by 36% compared to the control treatment (12.5%) [15].

3.3. Malondialdehyde Content

The highest amount of malondialdehyde was observed in the Polour, Fil Zamin, and German populations, with 38.45, 36.67, and 37.58 μmol per gram of fresh weight in the control treatment. The lowest levels of malondialdehyde, with values of 19.36, 20.14 and 23.69 μmol/g fresh weight, were obtained with the 100 μM salicylic acid treatment and in the Fil Zamin, Polour, and German populations, respectively. The results show that these values are 47.26, 47.62, and 36.96% lower than those for the control treatments, respectively, which shows that the 100 µM salicylic acid treatment has a very favorable effect in reducing the amount of malondialdehyde.

Malondialdehyde is used as a marker to assess lipid peroxidation and cell damage [16]. Various studies have investigated the effect of salicylic acid and methyl jasmonate elicitors on malondialdehyde levels. In chicory, treatment with 100 µM salicylic acid significantly reduced the amount of malondialdehyde compared to the control treatment [17]. It has been shown that treatment of the safflower (Carthamus tinctorius) cultivar of the Isfahan cultivar with 500 µM methyl jasmonate reduced the amount of malondialdehyde by 13.53% compared to the control treatment (1.6 mmol/g fresh weight), which showed that a reduction in the effects of stress is effected by methyl jasmonate treatment [18].

3.4. Proline Content

The highest levels of proline were observed in the Polour, Fil Zamin, and German populations, with 6.29, 6.33, and 7.15 µmol/g fresh weight with treatments of 100 µM salicylic acid, 100 µM salicylic acid, and 100 µM methyl jasmonate, respectively—38.33, 36.72 and 29.83% higher than the control treatment, respectively. The lowest amounts of proline in the Polour population, with the amount of 2.90 µmol/g fresh weight with the treatment of 200 µM salicylic acid + 200 µM methyl jasmonate; in the Fil Zamin population, with the amount of 2.75 µmol/g fresh weight with the treatment with 100 µM salicylic acid + 100 µM methyl jasmonate; and in the German population, with an amount of 2.69 µmol/g fresh weight obtained with the treatment of 200 µM salicylic acid + 200 µM methyl jasmonate, were 36.12, 40.60, and 48.57% lower than the control treatment, respectively.

As a non-toxic molecule with high solubility, the amino acid proline plays a vital role in maintaining the function of proteins, antioxidant enzymes, and osmotic regulation under a wide range of abiotic stresses, including salinity, drought, and stimulus stresses [19,20]. It has been shown that treatment with 1 mM salicylic acid in sage (Silybum marianum) caused a 35.56% increase in leaf proline content compared to the control treatment (42 mg/g fresh weight) [21]. In addition, the treatment of *Verbascum sinuatum* with 200 µM methyl jasmonate reduced the amount of proline compared to the control treatment (60 mg/g fresh weight) [11].

4. Conclusions

Studies show that salicylic acid and methyl jasmonate treatments improve stress-related indices well. The present study results show that foliar application of salicylic acid and methyl jasmonate at appropriate concentrations can increase the quantum efficiency of photosystem II, reduce ion leakage percentage and malondialdehyde content, and increase the proline content of leaves. It seems that the best treatments to increase plant capacity to deal with environmental stresses are 100 µM salicylic acid and 100 µM methyl jasmonate.

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