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

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

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1. Introduction

Persian poppy with the scientific name of *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]. It is mainly known as the major secondary metabolite in roots, leaves, and capsules due to its high accumulation of Thebaine, which is produced through the biosynthesis pathway of benzylisoquinoline. Other important drug alkaloids produced through this pathway include morphine, codeine, narcotine, oripavine, papaverine, noscapine, etc. [2]. Due to the non-narcotic and non-addictive nature of Thebaine and the ease of 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, where Australia, France, Hungary, Spain, and India had the largest share in production, respectively [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 rate have been revealed [5]. The use of methyl jasmonate in in-vitro cultures has been shown to activate antioxidant enzymes, express defense-related genes, and produce more 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 the 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 Damavand mountain on september by use of botanical information (*P. bracteatum* species has a straight stem without branching with a length of 50–80 cm with a single terminal flower, 3 to 8 brackets (its scientific name is due to its brackets), 4 to 6 bold red petals with one or two black spots in The base, a long, elongated capsule with short, dense hairs, can be identified on the sepals). For ease of reviewing the results, the population collected from the Polour region under the name of the first population, the next population with the accession number PAP 832 with Iranian origin, the Fil Zamin region under the name of the second population, and finally, the last population with the accession number PAP 754 with German origin, It is known as the third population.

The present experiment was based on a factorial experiment in the frame of 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 in four consequences times with 30 days intervals. Data analysis of biochemical properties of three different populations of Iranian poppy and Duncan's Multiple Range Test were performed using SPSS Statistics 26 (IBM) software Which is provided from DigiKala online store, Tehran, Iran.

2.1. Chlorophyll Fluorescence

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

2.2. Ion Leakage

Ion leakage was measured based on Sullivan & 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], And 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 at different levels of salicylic acid and methyl jasmonate, and their interaction was significantly different in all populations (level 1%) (Table 1).

Table 1. Results of variance analysis of stress-related indices among the population, salicylic acid and methyl jasmonate treatments of 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 the values of 0.835, 0.832, and 0.838, respectively, in the treatment of 100 μM salicylic acid, which was 14.86, 16.53, and 26.40 %, more than the control treatment respectively. 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 in 100 μM methyl jasmonate, 100 μM methyl jasmonate, and 200 μM salicylic acid treatments in the second, third and first populations, respectively. It shows that these amounts are 42.41, 49.54, and 40.68% less than the control treatments, respectively, which shows that 100 μM methyl jasmonate treatment in the second and third populations and 200 μM salicylic acid treatment significantly reduced ion leakage of leaves.

It has been shown that under stressful conditions, oxidation and alteration of the structure/nature of protein and lipid components of the membrane, by destroying its 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 treatment [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 و and

23.69 μmol/g fresh weight were obtained in 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% less than 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 treated with 100 μM salicylic acid significantly reduced the amount of malondialdehyde compared to the control treatment [17]. It has been shown that treatment of safflower (Carthamus tinctorius) cultivar of 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 a reduction in effects of stress is affected 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 in treatments of 100 μM salicylic acid, 100 μM salicylic acid, and 100 μM methyl jasmonate, respectively that 38.33, 36.72 and 29.83% are more than the control treatment, respectively. The lowest amount of proline in the Polour population with the amount of 2.90 μmol/g fresh weight in the treatment of 200 μM salicylic acid + 200 μM methyl jasmonate, the Fil Zamin population with the amount of 2.75 μmol/g fresh weight in the treatment of 100 μM salicylic acid + 100 μM Methyl jasmonate and the German population with an amount of 2.69 μmol/g fresh weight were obtained in the treatment of 200 μM salicylic acid + 200 μM methyl jasmonate, which are 36.12, 40.60 and 48.57% less 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 in a wide range of abiotic stresses, including salinity, drought, and stimulus stresses [19]. It has been shown that the treatment of 1 mM salicylic acid on sage (Silybum marianum) caused a 35.56% increase in leaf proline content compared to the control treatment (42 mg/g fresh weight) [20]. Also, 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 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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