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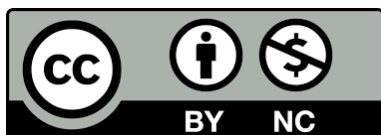
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# The effects of salicylic acid and melatonin elicitors on the antioxidant enzymes activity and morphological characteristics of *Acorus calamus* under drought stress

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**Abstract:** Medicinal plants improve human health, strengthen the immune system, reduce inflammation, and facilitate the treatment of diseases. To investigate the effect of drought stress (after 10 and 20 days of water withdrawal, representing mild and severe drought stress, respectively) and melatonin and salicylic acid on the biochemical and morphological indicators of the *Acorus calamus* medicinal plant, an experiment was conducted. Results showed that using melatonin and salicylic acid increased the activity of peroxidase enzyme under drought conditions. Also, salicylic acid increased the activity of catalase enzyme in mild drought stress. The application of melatonin and salicylic acid under severe drought stress increased the activity of the catalase enzyme. Under drought stress conditions, the use of salicylic acid and melatonin led to an increase in plant dry weight compared to normal conditions. Also using melatonin under severe drought conditions resulted in an increase in dry weight in comparison with the control treatment. In conclusion, while the plant's responses to stress and elicitors differed across various characteristics, it can generally be stated that salicylic acid and melatonin positively influence the biochemical and morphological traits of *Acorus calamus* under severe and mild drought stress conditions.

**Keywords:** Biochemical indicators, drought stress, foliar spraying, medicinal plant, morphological indicators.

## Introduction

Plants in natural conditions are subjected to various stresses that adversely affects their growth and production (Hosseini et al., 2019; Hosseini et al., 2021; Zulfiqar et al., 2021). Temperature, heavy metals, salinity, and water deficit are among the non-living factors that effectively affect the growth of plants. Among these factors, drought is the biggest factor limiting the production of agricultural products (Khalvandi et al., 2021). In other words, drought is one of the most important factors and the most common environmental stress that limits the growth and performance of plants worldwide (Keshavarz - Mirzamohammadi et al., 2021; Zulfiqar et al., 2021). Plants respond to drought stress at different physiological, cellular, and, molecular levels. This response depends on the species and genotype of the plant, the severity and duration of the water shortage, and the growth stage of the plant (Khalvandi et al., 2021; Zulfiqar et al., 2021; Ostadi et al., 2022).

*Acorus calamus* (Aghir in Turkish or small lily), is a plant that grows at riversides. This plant is valued as a "rejuvenator" for the brain and nervous system, as well as a remedy for digestive disorders. The leaves and rhizomes of this plant contain a volatile oil that has a special smell and taste (Rajput et al., 2014). The main components of the oil include beta- and alpha-asarone, saponins, lectins, sesquiterpenoids, lignans, and, steroids. Phytochemicals in plants vary based on geographic location, plant age, climate, species diversity, and extracted plant components (Rajput et al., 2014; Khalvandi et al., 2019; Hosseini et al., 2023).

In light of climate change and depletion of water resources, utilization of drought tolerant plant species or genotypes with low water demand represents an strategy for sustainable resource management and enhancing yield (Khalvandi et al., 2019; Haghpanah et al., 2024) The production of resistant plants requires a relatively long time from breeding methods, so the use of effective materials in reducing the adverse effects of stress to achieve the economic aspects of the performance of medicinal plants seems to be important (Khalvandi et al., 2019; González-Villagra et al., 2022; Khalil et al., 2022). Plants respond to different environmental conditions through changes in various biochemical

and physiological processes (Adl et al., 2023; Hosseini et al., 2023; Akbari et al., 2024). Plants can produce both volatile and non-volatile compounds including plant hormones that help them adapt to environmental changes (Kaur et al., 2022; Murali-Baskaran et al., 2022). Salicylic acid is a hormone-like substance that affects plant growth and development. Salicylic acid belongs to a group of plant phenols (Zulfiqar et al., 2021). This compound plays an important role in regulating plant growth and development, interacting with other organisms, responding to environmental stresses, glycolysis, photosynthetic performance, transpiration and fruiting (González-Villagra et al., 2022). Various studies have shown that salicylic acid significantly causes ion leakage and reduces the accumulation of toxic ions in plants (Khalvandi et al., 2021; Zulfiqar et al., 2021; González-Villagra et al., 2022). According to the reports, salicylic acid has significant effects on the morphological and physiological characteristics of the plant and plays a role in stimulating the support mechanisms to increase resistance against biotic and abiotic stresses (Khalvandi et al., 2021).

Melatonin is another compound that plays an important role in regulating the physiological and biochemical activities of plants (Murch and Erland, 2021). Melatonin plays a significant role in helping plants cope with stress by regulating physiological and biochemical responses (Ali et al., 2024). It enhances antioxidant activity, protects cellular structures, and improves water retention, thus promoting plant growth under adverse conditions (Ali et al., 2024; Cano et al., 2024). Additionally, melatonin influences the production and synthesis of secondary metabolites in medicinal plants, such as flavonoids and phenolics (Ghorbani et al., 2024a). These compounds contribute to plant defense mechanisms and enhance medicinal properties, making melatonin crucial for improving both stress tolerance and therapeutic potential in medicinal herbs (Back, 2021; Murch and Erland, 2021; Ali et al., 2024; Cano et al., 2024; Ghorbani et al., 2024b).

Given the limited understanding of plant hormones, particularly melatonin, in plant responses to drought stress, it is essential to conduct extensive research on the effects of this hormone. Therefore, the present study was conducted to investigate the effects of drought stress and

exogenous application of salicylic acid and melatonin on the morphological and biochemical characteristics of the *Acorus calamus* medicinal plant.

## Materials and Methods

### *Plant materials and treatments*

The current research was conducted using two factors factorial experiment based on a completely randomized design with three replications. The first factor included two levels the drought stress, continuous irrigation (W) and, interruption of irrigation (No-W). The second factor included foliar spraying of elicitors at three levels (distilled water (control), melatonin (ML) (100  $\mu$ M), and, salicylic acid (SA) (1 mM). Foliar sprays were carried out once every 5 days until harvest time. The first foliar spraying was done when stopping the irrigation.

The samples of *Acorus calamus* along with the rhizome were collected from Arzefoon wetland near Sari City, Mazandaran province, and, transferred to the greenhouse. Plants were planted on separate beds with dimensions of 40 x 200 cm, which were isolated using plastic covers. These beds were prepared with river sand plus garden soil with a ratio of 1:1 to a depth of 15 cm. The conditions for the growth of plants were provided by flooding. Each plot contained 15 plant samples. The studied plants were kept under these conditions for two months under solar light irradiance of 60000 lux, the temperature of 30-35 °C and, relative humidity of 70 % in greenhouse conditions.

### *Traits measurement*

Sampling was done at two time points: 10 and 20 days after water withdrawal, representing mild and severe drought stress, respectively. After recording the morphological traits, the samples were transferred to -80 °C for biochemical measurements. Characters including fresh and dry weight, number of roots, length of roots, number of nodes in the rhizome and number of leaves were measured from these samples. To calculate the dry weight, the samples were kept at 76 °C for 72 hours and then weighed.

### *Extraction of enzyme and soluble protein*

Fresh tissue was weighed and homogenized with 2 ml of potassium phosphate buffer (pH=6.8). After

homogenization, the samples were transferred to two ml vials and centrifuged at 4°C at 15,000 rpm for 12 minutes. Then, the upper phase was separated and these extracts were used for enzyme assays. The Bradford method (Bradford, 1976) was used to measure soluble protein concentration.

### *Catalase (CAT) enzyme activity assay*

The Luck method (Luck, 1971) was used to measure CAT enzyme activity. 20  $\mu$ l of enzyme extract was mixed with 980  $\mu$ l of phosphate buffer containing 2 mM hydrogen peroxide and their absorbance changes were read at a wavelength of 240 nm using a spectrophotometer. The enzyme activity was calculated using the Beer-Lambert law and an extinction coefficient of 40 ( $\mu$ M<sup>-1</sup>cm<sup>-1</sup>). The enzyme activity was calculated in terms of mmol per minute per mg of protein.

### *Superoxide dismutase enzyme activity assay*

Superoxide dismutase (SOD) enzyme activity was measured using the nitroblue tetrazolium (NBT) photoreduction inhibition assay at a wavelength of 560 nm. For this purpose, 50 mM potassium phosphate buffer solution (pH=7.4) was prepared. Then, 13 mM methionine, 0.1 mM EDTA, 75  $\mu$ M NBT, and 4  $\mu$ M riboflavin were added to prepare the reaction mixture, respectively. Before adding these compounds to the potassium phosphate buffer, we covered the container containing the reaction mixture so that it was not exposed to light. Then, 100  $\mu$ l of extract from each sample was poured into each test tube and 3 ml of the above solution was added to it and the reaction was started immediately by placing them under the light of a fluorescent lamp (W40). After 1.5 minutes, the absorbance of the samples was read at a wavelength of 560 nm.

The activity of the SOD enzyme in the samples was measured in comparison with the light control. The difference in absorbance of the samples and the light control at 560 nm indicates the inhibition of the photoreduction of NBT in the presence of the SOD enzyme present in the sample. Using this absorbance difference, the enzyme activity was calculated in terms of enzyme units per gram of fresh weight for all samples (Beauchamp and Fridovich, 1971).

### ***Peroxidase (POD) enzyme activity assay***

POD was assayed using the method of (Nakano and Asada, 1981). The reaction mixture consisted of 2.5 ml of 50 mM phosphate buffer (pH=7.0) containing 0.1 mM EDTA, 1 mM sodium ascorbate, 0.2 ml of 1% H<sub>2</sub>O<sub>2</sub>, and 0.1 ml of extracted protein extract. POD was calculated as the decrease in absorbance at 240 nm for 1 min (Extinction coefficient= 2.18 mM<sup>-1</sup> cm<sup>-1</sup>).

### ***Statistical analysis***

The extracted data were first normalized and then analyzed using SAS (9.1) statistical software. Comparisons of mean data were performed using the least significant difference (LSD) method at a 5 % probability level, and figures were drawn using EXCEL software. Also, XLSTAT (2016) software was used to perform principal component analysis and cluster analysis. The Varimax rotation method was also used to maximize high- and low-value factor loadings and minimize mid-value factor loadings. Euclidean distance and the UPGME method were used for cluster analysis.

## **Results**

### ***POD enzyme activity***

Analysis of variance showed that the effects of drought stress, elicitor and their interaction were significant for the POD at mild and severe drought stress ( $P \leq 0.01$ ) (Table S1). The results of mild drought stress demonstrated that the highest amount of POD was observed in No-W+SA (13.67 U/mg protein min) and No-W+ML treatments (15 U/mg protein min) and the lowest amount of POD was observed in W (normal) treatments (6.67 U/mg protein min), W+ML (5 U/mg protein min) and No-W (6 U/mg protein min) (Figure 1). The use of W+SA, No-W+ML, and No-W+SA treatments increased the POD by 60, 125, and 105 %, respectively, in comparison with W treatment (Figure 1). In severe drought stress, the highest amount of POD was observed in the No-W+SA treatment (47.12 U/mg protein min) (Figure 1). On the other hand, the lowest amount of POD was observed in W (7.05 U/mg protein min) and No-W (7.04 U/mg protein min) treatments, which showed no statistically significant difference with W+ML (14.33 U/mg protein min) and No-W (7.04 U/mg protein min) treatments. Using W+SA and No-

W+SA treatments caused 197 and 568 % increases in POD in comparison with W treatment, respectively (Figure 1).

### ***CAT enzyme activity***

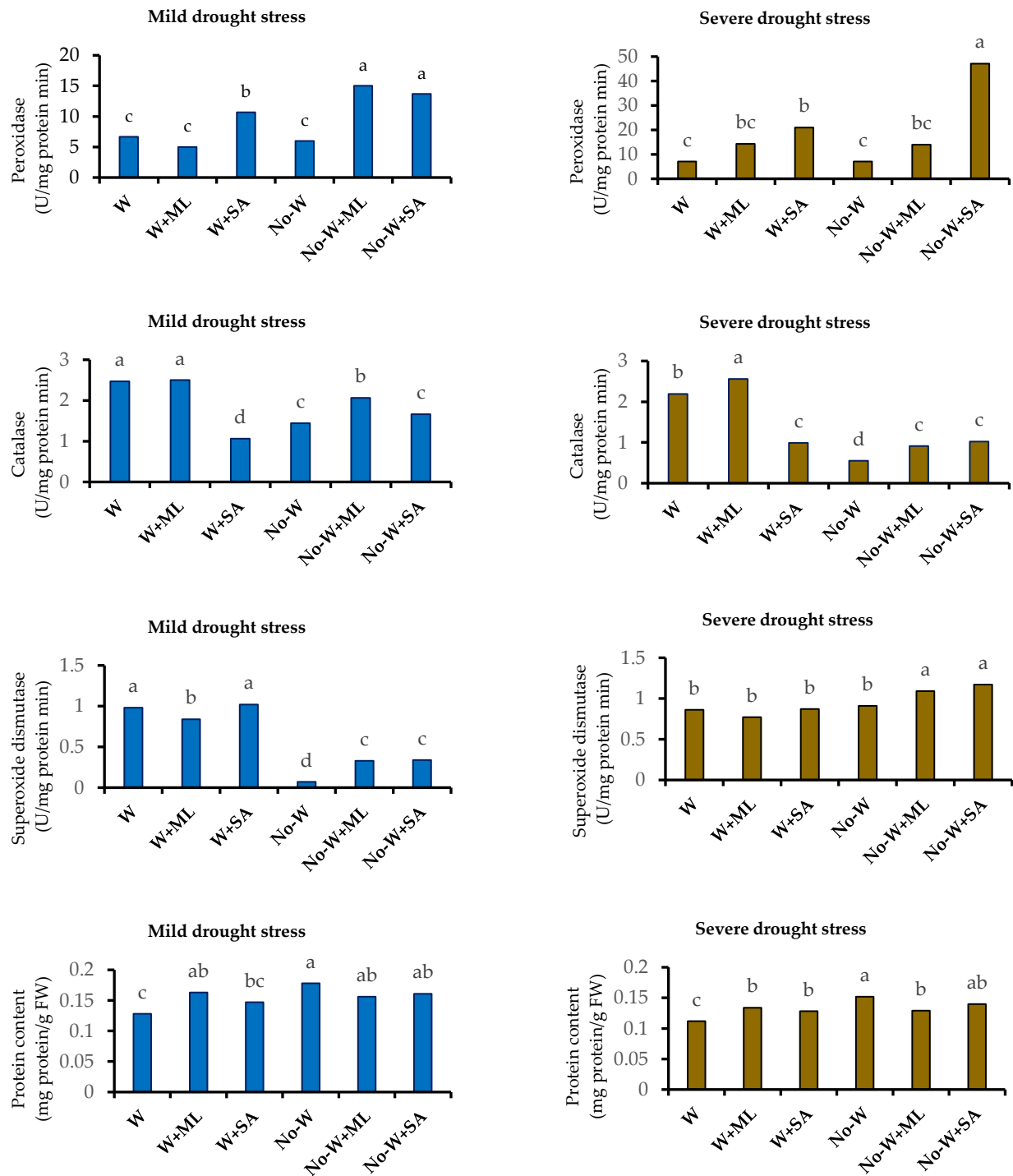
According to analysis of variance, the CAT was significantly affected by drought stress, elicitors, and their interaction at mild and severe drought stress exposure ( $P \leq 0.01$ ) (Table S1). In mild drought stress exposure the highest amount of CAT was observed in W (2.47 U/mg protein min) and W+ML (2.5 U/mg protein min) treatments (Figure 1), however, the lowest amount of CAT was observed in W+SA treatment (1.06 U/mg protein min). Application of W+SA, No-W, No-W+ML, and No-W+SA treatments resulted in a reduction of 133, 71, 20, and 49 % for CAT in comparison with W treatment, respectively (Figure 1).

In severe drought stress, the highest amount of CAT was observed in the W+ML treatment (2.56 U/mg protein min) (Figure 1), but the lowest amount belonged to the No-W treatment (0.55 U/mg protein min). Application of W+ML treatment caused a 17 % increase in CAT in comparison with W treatment; but using W+SA, No-W, No-W+ML, and No-W+SA treatments caused decreased 121, 298, 141, and 115 % of CAT in comparison with W treatment, respectively (Figure 1).

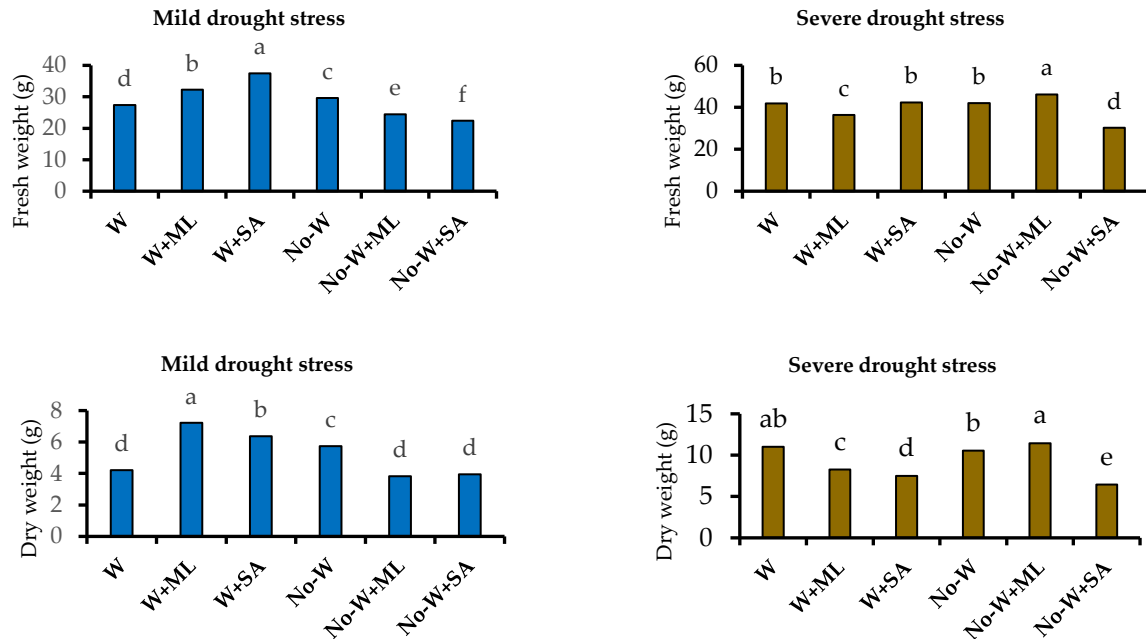
### ***SOD enzyme activity***

Results showed that the effects of drought stress ( $P \leq 0.01$ ), elicitor ( $P \leq 0.05$ ), and their interaction ( $P \leq 0.01$ ) were significant for SOD at mild and severe drought stress (Table S1).

Ten days after stress exposure illustrated that the highest amount of SOD was observed in W (0.98 U/mg protein min) and W+SA (1.02 U/mg protein min) treatments, however, the lowest amount of SOD belonged to No-W treatment (0.07 U/mg protein min) (Figure 1). In severe drought stress, the highest amount of SOD was observed in No-W+ML (1.09 U/mg protein min) and No-W+SA (1.17 U/mg protein min) treatments (Figure 1). However, the lowest activity of SOD was correlated with W (0.86 U/mg protein min), W+ML (0.77 U/mg protein min), W+SA (0.87 U/mg protein min), and No-W (0.91 U/mg protein min) treatments. Application of No-W+ML and No-W+SA treatments promoted 27 and 36 % SOD in comparison with W treatment, respectively (Figure 1).



**Figure 1.** The effects of foliar spraying of salicylic acid (SA) and melatonin (ML) on the activity of antioxidant enzymes and protein content in *Acorus calamus* plant under continuous irrigation (W) and mild and severe drought stress (No-W). Continuous irrigation (W), continuous irrigation plus application of melatonin (W+ML), continuous irrigation plus application of salicylic acid (W+SA), mild and severe drought stress (No-W), mild and severe drought stress plus application of melatonin (No-W+ML), mild and severe drought stress plus application of salicylic acid (No-W+SA). The blue and brown charts correspond to the mild and severe drought stress, respectively.



**Figure 2.** The effect of foliar spraying of salicylic acid (SA) and melatonin (ML) on the fresh and dry weight of the *Acorus calamus* plant under the treatment of continuous irrigation (W) and mild and severe drought stress (No-W). Continuous irrigation (W), continuous irrigation plus application of melatonin (W+ML), continuous irrigation plus application of salicylic acid (W+SA), mild and severe drought stress (No-W), mild and drought stress plus application of melatonin (No-W+ML), mild and severe drought stress plus application of salicylic acid (No-W+SA).

### Protein content

The results showed that the protein content in mild and severe drought stress was significantly under the influence of drought stress ( $P \leq 0.05$ ) (Table S1), however, using elicitors demonstrated cannot significantly increase the protein content. The interaction of drought stress and elicitor changed significantly ( $P \leq 0.05$ ) the protein content (Table S1). In mild drought stress, the highest amount of protein content was observed in No-W treatment (0.178 mg protein/g FW), when compared with W+ML (0.163 mg protein/g FW), No-W+ML (0.156 mg protein/g FW) and No-W+SA treatments (0.161 mg protein/g FW), illustrating no significant difference (Figure 1). In contrast, the lowest amount of protein content was related to W treatment (0.128 mg protein/g FW), which showed no significant difference with W+SA treatment (0.147 mg protein/g FW). Results showed that using W+ML, No-W, No-W+ML and No-W+SA treatments increased the amount of protein content by 27, 39, 22 and 26 %, respectively, in comparison with the W treatment (Figure 1).

The results related to the severe drought stress showed that the highest amount of protein content was achieved by the application of No-W treatment (0.152 mg protein/g FW), which had no statistically significant difference with the No-W+SA treatment (0.14 mg protein/g FW) (Figure 1). On the other hand, the lowest amount of protein content was observed in the W treatment (0.112 mg protein/g FW). The results showed that the use of W+ML, W+SA, No-W, No-W+ML and No-W+SA treatments increased the amount of protein content by 19, 14, 35, 15 and 25 %, respectively, in comparison with the W treatment (Figure 1).

### Fresh and dry weight

Analysis of variance showed that the fresh and dry weight in the time of mild drought stress was significantly affected by drought stress ( $P \leq 0.01$ ), elicitors ( $P \leq 0.01$ ) and their interaction ( $P \leq 0.01$ ) (Table S2, Figure 2). On the other hand, the drought stress had no significant effect on the fresh weight in the severe drought stress. The results of the analysis of variance showed that the fresh weight in the severe drought stress was significantly affected

by elicitors ( $P \leq 0.01$ ) and the interaction of drought stress and elicitors ( $P \leq 0.01$ ). The results of the analysis of variance showed that the dry weight in the severe drought stress was significantly changed by drought stress ( $P \leq 0.01$ ), elicitors ( $P \leq 0.01$ ) and, their interaction ( $P \leq 0.01$ ) (Table S2).

The results of mild drought stress revealed that the highest amount of fresh weight was related to the W+SA treatment (37.44 g) and the lowest amount of fresh weight was related to the No-W+SA treatment (22.41 g) (Figure 2). The application of No-W, W+ML and W+SA treatments increased the amount of fresh weight by 8, 21 and, 36 %, respectively, compared to the W treatment. In contrast, the application of No-W+ML and No-W+SA treatments decreased the fresh weight by 12 and 22 %, respectively, in comparison with the W treatment (Figure 2).

In severe drought stress the heaviest fresh weight was produced by the No-W+ML treatment (46.18 g) (Figure 2) and the lightest was obtained from the No-W+SA treatment (30.24 g). According to the results using No-W+ML treatment created 10 % more fresh weight from the W treatment; while the application of W+ML and No-W+SA treatments compared to the W treatment caused 15 and 38 % reduction in fresh weight, respectively (Figure 2).

In mild drought stress, the most dry weight was observed in the W+ML treatment (7.23 g); In contrast, the least dry weight were measured in W (4.21 g), No-W+ML (3.83 g) and No-W+SA (3.94 g) treatments. The W+ML, W+SA and No-W treatments were able to make 71, 51 and 36 % more dry weight, respectively (Figure 2). Furthermore, the severe drought stress showed the highest dry weight in the No-W+ML treatment (11.45 g), which had no significant difference with the W treatment (11 g) (Figure 2). However, the lowest dry weight was obtained from the No-W+SA treatment (6.46 g). Using W+ML, W+SA and No-W+SA treatments compared to the W treatment have yielded 33, 46 and 70 % reduction in dry weight, respectively (Figure 2).

#### *Number of leaves*

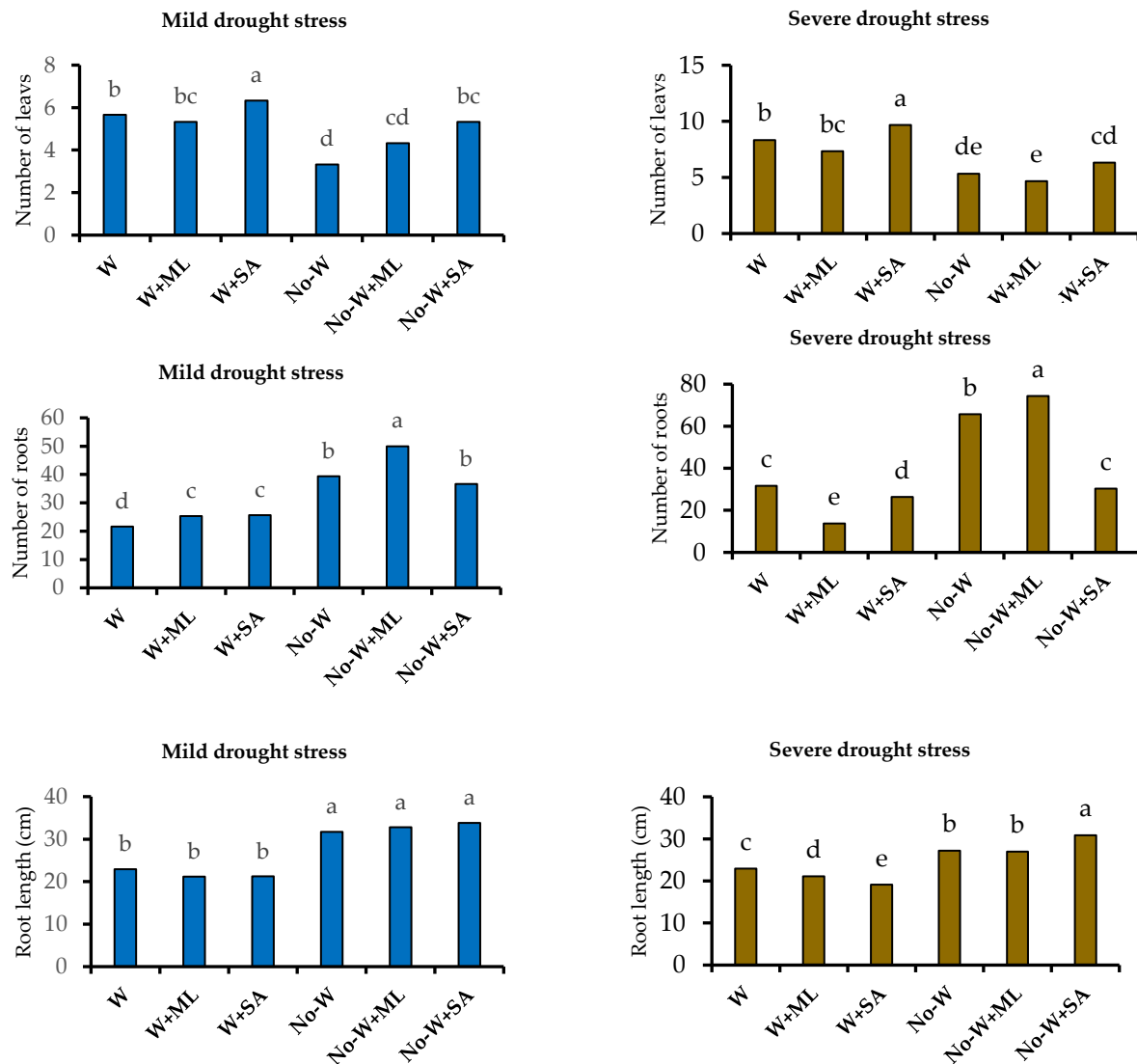
The results related to the conditions of mild drought stress showed that the highest number of leaves was

achieved in the W+SA treatment (7.33) (Figure 3). On the other hand, the lowest number of leaves was achieved in the No-W treatment (3.33), which was not statistically significantly different from the No-W+ML treatment (7.33). The results showed that the W+SA treatment increased the number of leaves by 29 % compared to the W treatment. Based on the obtained results, the use of No-W and No-W+ML treatments reduced the number of leaves by 70 % and 31 %, respectively compared to the W treatment (Figure 3). The results indicated that the W+SA treatment produced the highest number of leaves (9.67), while the No-W+ML treatment had the lowest (4.67), with no significant difference from the No-W treatment (5.33). Also, the application of W+SA treatment caused a 16 % increase in the number of leaves compared to the W treatment. Conversely, the application of No-W, No-W+ML and No-W+SA treatments resulted in decrease in the number of leaves by 56, 78 and 31 %, respectively, compared to the W treatment (Figure 3)

#### *Root length*

According to the analysis of variance, root length at mild drought stress was significantly influenced by drought stress ( $P \leq 0.01$ ) and the interaction of drought stress and elicitors ( $P \leq 0.01$ ) (Table S2). The highest root lengths were observed in the No-W (31.74 cm), No-W+ML (32.77 cm) and No-W+SA (33.8 cm) treatments, while the lowest were in the W (22.91 cm), W+ML (21.18 cm), and W+SA (21.24 cm) treatments (Figure 3). The No-W, No-W+ML, and No-W+SA treatments increased root length by 38, 43 and 47%, respectively, compared to the W treatment (Figure 3).

At severe drought stress, the analysis of variance indicated significant effects from drought stress ( $P \leq 0.01$ ), elicitors ( $P \leq 0.01$ ) and their interaction ( $P \leq 0.01$ ). The highest root length was achieved with the No-W+SA treatment (30.9 cm), while the lowest was in the W+SA treatment (19.1 cm). The No-W, No-W+ML, and No-W+SA treatments increased root length by 18, 17 and 34%, respectively, compared to the W treatment. Conversely, the W+ML and W+SA treatments resulted in decreases of 8 and 20% in root length, respectively, compared to the W treatment (Figure 3).



**Figure 3.** The effect of foliar spraying of salicylic acid (SA) and melatonin (ML) on the number of roots and leaves and root length of the *Acorus calamus* plant under the treatment of continuous irrigation (W) and mild and severe drought stress (No-W). Continuous irrigation (W), continuous irrigation plus application of melatonin (W+ML), continuous irrigation plus application of salicylic acid (W+SA), mild and severe drought stress (No-W), mild and severe drought stress plus application of melatonin (No-W+ML), mild and severe drought stress plus application of salicylic acid (No-W+SA).

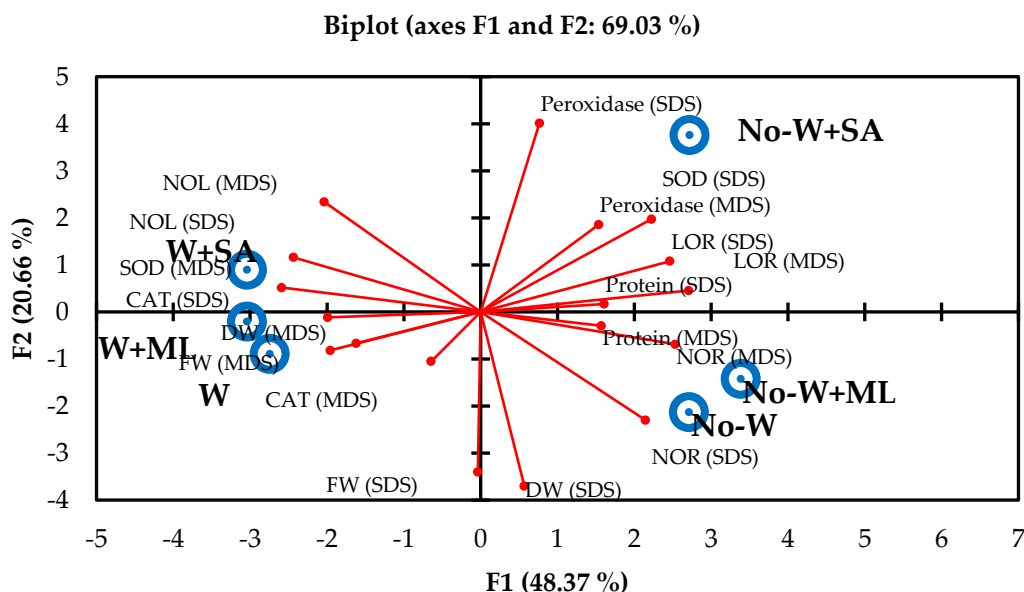
### Principal components analysis (PCA) and cluster analysis (CA)

Based on the results obtained from the PCA, it was observed that the four\_components had an eigenvalue higher than one and were known as effective components (Table 1). The first and second components had a relative variance of 48.37 and 20.65 %, respectively, and accounted for 69.02 % of the total variance (Table 1). The results showed that

in the first component, SOD, fresh weight, number of roots, number of leaves and root length traits in the mild drought stress and CAT, SOD, number of roots, number of leaves and root length traits had high factor loadings at the severe drought stress (Table 1). Also, in the second component, POD, fresh weight and dry weight traits had high factor loading (Table 1).

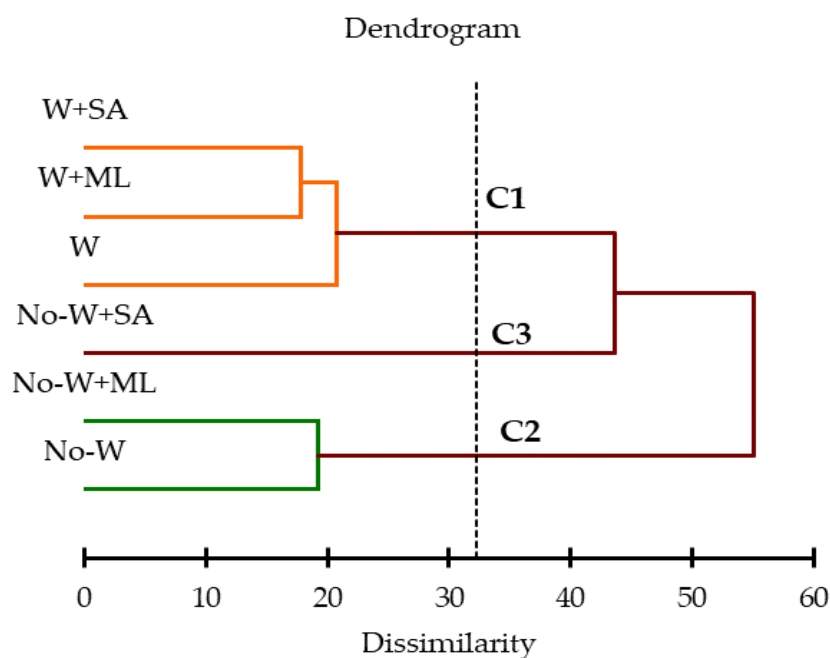
**Table 1.** Factor loading related to different traits in *Acorus calamus* plants under salicylic acid treatments and drought stress (Mild and severe drought stress).

Characteristics	Factors				
	F1	F2	F3	F4	F5
Peroxidase (Mild drought stress)	0.561	0.443	-0.388	-0.473	0.339
Catalase (Mild drought stress)	-0.235	-0.251	-0.530	0.741	0.227
Superoxide dismutase (Mild drought stress)	-0.945	0.124	-0.252	-0.147	0.080
Protein (Mild drought stress)	0.573	-0.070	0.766	0.221	0.173
Peroxidase (Severe drought stress)	0.280	0.957	0.030	-0.004	0.064
Catalase (Severe drought stress)	-0.726	-0.028	-0.270	0.607	0.175
Superoxide dismutase (Severe drought stress)	0.812	0.471	-0.312	-0.141	0.045
Protein (Severe drought stress)	0.588	0.040	0.796	0.138	-0.029
Fresh weight (Mild drought stress)	-0.715	-0.195	0.534	-0.399	0.078
Dry weight (Mild drought stress)	-0.590	-0.159	0.753	0.069	0.232
Number of roots (Mild drought stress)	0.925	-0.164	-0.037	-0.142	0.311
Number of leaves (Mild drought stress)	-0.744	0.558	-0.265	-0.239	0.089
Length of roots (Mild drought stress)	0.989	0.108	-0.053	0.042	-0.073
Fresh weight (Severe drought stress)	-0.013	-0.813	-0.221	-0.528	0.106
Dry weight (Severe drought stress)	0.208	-0.883	-0.410	0.012	-0.089
Number of roots (Severe drought stress)	0.784	-0.549	-0.113	-0.268	-0.009
Number of leaves (Severe drought stress)	-0.889	0.276	0.006	-0.308	-0.194
Length of roots (Severe drought stress)	0.900	0.257	-0.123	0.268	-0.193
Eigenvalue	8.707	3.719	3.060	2.011	0.504
Variability (%)	48.370	20.658	17.000	11.173	2.799
Cumulative (%)	48.370	69.028	86.028	97.201	100.000

**Figure 4.** Bi-plot resulting from PCA based on all studied traits in *Acorus calamus* plants under the exposure of drought stress and application of elicitors treatments. (Mild drought stress (MDS); severe drought stress (SDS); catalase (CAT); superoxide dismutase (SOD); continuous irrigation (W), continuous irrigation plus application of melatonin (W+ML), continuous irrigation plus application of salicylic acid (W+SA), mild and severe drought stress (No-W), mild and severe drought stress plus application of melatonin (No-W+ML), mild and severe drought stress plus application of salicylic acid (No-W+SA).

The bi-plot diagram obtained from the first and second components showed that No-W+SA treatment had a high correlation with POD, SOD, root length and protein traits in the severe drought stress and POD and root length traits in the mild drought stress (Figure 4). The No-W+SA treatment was separated independently due to the large distance compared to other treatments. On the other hand, the W+SA, W+ML and W treatments were placed in the same group due to their high affinity and were correlated with number of leaves, SOD, dry weight, fresh weight and CAT traits in the mild drought stress and number of leaves and CAT traits

in the severe drought stress. Also, No-W+ML and No-W treatments were placed in the same group and showed a strong correlation with the number of roots and protein content traits in the mild drought stress and fresh weight, dry weight and, number of roots traits in the severe drought stress (Figure 4). The results of the cluster analysis showed that all the investigated treatments were divided into three separate groups, so in the first group there were three treatments (W+SA, W+ML and, W), in the second group there were two treatments (No-W+SA and No-W+ML) and in the third group one treatment (No-W) were separated (Figure 5).



**Figure 5.** Dendrogram resulting from cluster analysis based on all studied traits in *Acorus calamus* plants under the exposure of drought stress and application of elicitors treatments. Continuous irrigation (W), continuous irrigation plus application of melatonin (W+ML), continuous irrigation plus application of salicylic acid (W+SA), mild and severe drought stress (No-W), mild and severe drought stress plus application of melatonin (No-W+ML), mild and severe drought stress plus application of salicylic acid (No-W+SA).

## Discussion

Drought stress is one of the most important limiting factors for plant growth and changes the physiological activities of plants (Khalvandi et al., 2021). Among these, elicitors play an important role in regulating the physiological activities of plants

under environmental stress conditions, especially drought stress (Moustafa-Farag et al., 2020). In the present experiment, the use of salicylic acid and melatonin led to significant changes in the morphological and physiological characteristics of the *A. calamus* L. plant.

When plants encounter drought stress, the amount of reactive oxygen species (ROS) in their cells increases. This rise in ROS leads to damage to cell membranes and the degradation of biological molecules. Therefore, the removal of ROS is one of the key strategies for plants to tolerate drought stress in (Zulfiqar *et al.*, 2021). In our experiment, the response of antioxidant enzymes to drought stress was different; in most cases, the activity of these enzymes decreased with the application of drought stress. Additionally, the activity of antioxidant enzymes in response to environmental stress differ across various plant organs (Pan *et al.*, 2021).

In studies conducted on mint plants (Khalvandi *et al.*, 2019; Hosseini *et al.*, 2023), basil (Zulfiqar *et al.*, 2021), thyme (Rahimi *et al.*, 2022) and rosemary (Mohammadi-Cheraghabadi *et al.*, 2021; Ostadi *et al.*, 2022), an increase in the activity of antioxidant enzymes in response to salinity and drought stress has been reported, which support the findings of our study. CAT, ascorbate POD and SOD are among the most effective antioxidant enzymes. The increased activity of these enzymes enhances tolerance to various stresses, including drought stress, in plants (Khalvandi *et al.*, 2019; Hosseini *et al.*, 2023).

On the other hand, the results of the present experiment indicated that the use of salicylic acid and melatonin increased the activity of antioxidant enzymes under both normal conditions and drought stress, however, the response to these elicitors varied somewhat differently under normal conditions.

Salicylic acid not only enhances antioxidant activity but also reduces the toxicity of free radicals that have accumulated due to drought stress damage, and thus reduces the damage caused by drought stress (González-Villagra *et al.*, 2022). Additionally, the application of salicylic acid increases the content of polyamines, which play an important role in developing tolerance to drought stress (Hasan *et al.*, 2021). Foliar spraying of wheat plant with salicylic acid has enhanced drought stress tolerance by increasing membrane stability, reducing leakage and peroxidation of cell membrane lipids (Khalvandi *et al.*, 2021).

Salicylic acid induces metabolic changes that increase ROS levels and enhance the antioxidant

defense system, playing a role in cellular signaling under drought stress conditions (Salvi *et al.*, 2021). By activating the enzymes involved in the biosynthesis of antioxidant enzymes, salicylic acid facilitates the removal and inactivation of ROS. Consequently, by enhancing the levels of both enzymatic and non-enzymatic antioxidants, it potentially reduces hydrogen peroxide in plant cells by ascorbate as a very important substrate for hydrogen peroxide detoxification in the photosynthetic apparatus (Hayat *et al.*, 2021).

Increasing antioxidant activity is a crucial important mechanism by which plants enhance their tolerance to environmental stresses, including drought. The exogenous application of salicylic acid significantly reduced malondialdehyde levels in wheat plants subjected to drought stress (Khalvandi *et al.*, 2021). In another study, the application of salicylic acid resulted in decreased malondialdehyde production, indicating the ability of this plant growth regulator to neutralize ROS and prevent their accumulation (Damalas and Koutroubas, 2021).

Probably, salicylic acid reduced the content of superoxide and hydrogen peroxide radicals by influencing the activity of antioxidant enzymes and inhibited the activity of lipoxygenase enzyme and the breakdown of membrane fatty acids, ultimately leading to a reduction in oxidative damage to membrane lipids during drought stress (Damalas and Koutroubas, 2021; Khalvandi *et al.*, 2021). Research has demonstrated that salicylic acid acts as a resistance mechanism by boosting the antioxidant capacity of cells. It provides greater protection to cell membranes, reduces lipid peroxidation, and prevents protein oxidation (Kaur *et al.*, 2022). In an experiment with tomato plants, phenolic compounds accumulated after the application of salicylic acid following the increase in the activity of the phenylalanine ammonia-lyase enzyme (Fan *et al.*, 2022). Therefore, salicylic acid plays an important role in the biosynthesis of phenolic compounds and the expression of plant defense genes (Khalil *et al.*, 2022). The results of the present experiment demonstrated the positive effect of melatonin on some morphological and physiological characteristics. Probably, melatonin stimulates plant growth by affecting plant metabolites and increasing the biosynthesis of

phytohormones such as auxin and cytokinin, and facilitating nutrient absorption (Tiwari *et al.*, 2021). By increasing turgor pressure and the relative water content of leaves, melatonin improves growth under drought stress conditions (Moustafa-Farag *et al.*, 2020).

According to the results of the present experiment, drought stress reduced the number of leaves, however, the application of melatonin and salicylic acid increased the number of leaves under stress conditions. Conversely, while melatonin and salicylic acid enhanced dry weight under normal conditions, they did not have a positive effect on dry weight under drought stress. It seems that under drought stress, the composition of melatonin affects the secondary metabolites involved in the stress resistance mechanism to maintain the plant survival, but does not affect the increase in dry matter (Tiwari *et al.*, 2021). How elicitors affect physiological processes in plants is very complex and requires further investigations in biological systems (Altaf *et al.*, 2022). The positive effect of melatonin on photosynthetic indices is proven in various plants under drought stress such as chickpea (Ahmad *et al.*, 2023), chrysanthemum (Luo *et al.*, 2023) and barley (Talaat, 2023).

Drought stress causes stomatal closure and reduced internal CO<sub>2</sub>, decreasing NADPH demand in the Calvin cycle, leading to oxidation and disruption of the electron transport chain, using oxygen as an electron acceptor and generating singlet oxygen. Previous studies indicate that melatonin mitigates drought stress effects on photosynthesis by maintaining light energy balance (Khalvandi *et al.*, 2021; Karumannil *et al.*, 2023; Zahra *et al.*, 2023).

Excess light energy triggers the Mehler reaction, producing superoxide and hydrogen peroxide, which oxidize membrane lipids and damage photosynthetic pigments, reducing photosynthesis. Melatonin exogenously protects chlorophyll by reducing lipid peroxidation and regulating chlorophyll-degrading genes (Zahra *et al.*, 2023). Melatonin enhances chloroplast levels, improves the Fv/Fm ratio, and prevents chloroplast damage (Rehaman *et al.*, 2021; Karumannil *et al.*, 2023). Melatonin also increases compatible osmolytes and phenol, improving turgor pressure and protecting cellular structures during stress (Moustafa-Farag *et al.*, 2020; Khalid *et al.*, 2022). Additionally,

melatonin reduces hydrogen peroxide and ion leakage, enhancing plant survival under drought stress (Tiwari *et al.*, 2021).

When oxidative damage occurs, plants activate antioxidant systems. Melatonin enhances H<sup>+</sup>-ATPase pump activity by converting to 5-methoxytryptamine, stabilizing the plasma membrane during stress (Siddiqui *et al.*, 2021). Additionally, phenolic and flavonoid hydroxyl groups inhibit free radicals, protecting cell membranes (Jafari and Shahsavari, 2021). On the other hand, in our experiment, root length increased under drought stress. It seems that plants increase the length of their roots to escape from drought stress conditions, so that they can overcome the stress limitation by increasing the absorption of water and nutrients (Ranjan *et al.*, 2022).

## Conclusion

The results of the present experiment showed that salicylic acid and melatonin had a positive effect on the activity of different antioxidant enzymes such as CAT, SOD, and POD, at mild and severe drought stress. The positive effect of these elicitors on the enzyme activity was also significant in the absence of drought stress. Additionally, salicylic acid and melatonin significantly increased the dry weight of the plant in mild drought stress. The number of roots increased with the application of these elicitors at this treatment. The application of salicylic acid increased root length, while melatonin increased the number of roots. Finally, it can be concluded that although the plant's response to stress and elicitors varied in terms of different characteristics, generally, salicylic acid and melatonin have a positive effect on the biochemical and morphological characteristics of the *Acorus calamus* medicinal plants under mild and severe drought stress.

## Supplementary Materials

The supplementary material for this article can be found online at: [https://www.jpmb-gabit.ir/article\\_721544.html](https://www.jpmb-gabit.ir/article_721544.html).

Supplementary Table S1. Supplementary Figure 1. Analysis of variance related to antioxidant enzyme activity and protein content traits in response to mild and severe drought stress and elicitors treatments.

**Supplementary Table S2.** Analysis of variance of fresh weight (FW), dry weight (DW), number of roots (NOR), number of leaves (NOL), length of roots (LOR) and number of internodes (NOIN) in response to mild and severe drought stress.

### Author Contributions

Conceptualization, G.R. and A.A.; methodology, A.A.; software, S.J.H.; validation, G.R., V.G. and A.A.; formal analysis, S.J.H.; investigation, A.A.; resources, G.R.; data curation, G.R.; writing—original draft preparation, S.J.H.; writing—review and editing, G.R.; visualization, V.O.; supervision, G.R.; project administration, G.R.; funding

acquisition, G.R. All authors have read and agreed to the published version of the manuscript.

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### Conflict of Interest Statement

The authors declare no conflict of interest.

### References

- Adl, S., Masoudian, N., Roudi, B., and Ebadi, M. (2023). Response of wheat to drought stress: focus on root and shoot nutrients, as well as leaf chlorophyll and glycine betaine. *J Plant Mol Breed.* 11(2): 39-54.
- Ahmad, R., Alsahli, A.A., Alansi, S., and Altaf, M.A. (2023). Exogenous melatonin confers drought stress by promoting plant growth, photosynthetic efficiency and antioxidant defense system of pea (*Pisum sativum* L.). *Sci. Hort.* 322: 112431.
- Akbari, A., Abbasian, A., Abbasi, R., and Zafarian, F. (2024). The effects of *Trichoderma* fungi symbiosis and nitrogen on essential oil and leaf pigments in green cumin (*Cuminum cyminum* L.) under weed competition. *J Plant Mol Breed.* 12(1): 87-106.
- Ali, M.A., Nasser, M.A., Abdelhamid, A.N., Ali, I.A., Saady, H.S., and Hassan, K.M. (2024). Melatonin as a key factor for regulating and relieving abiotic stresses in harmony with phytohormones in horticultural plants—a review. *J. Soil Sci. Plant Nutr.* 24(1): 54-73.
- Altaf, M.A., Shahid, R., Ren, M.-X., Naz, S., Altaf, M.M., Khan, L.U., Tiwari, R.K., Lal, M.K., Shahid, M.A., and Kumar, R. (2022). Melatonin improves drought stress tolerance of tomato by modulating plant growth, root architecture, photosynthesis, and antioxidant defense system. *Antioxid.* 11(2): 309.
- Back, K. (2021). Melatonin metabolism, signaling and possible roles in plants. *Plant J.* 105(2): 376-391.
- Beauchamp, C., and Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* 44(1): 276-287.
- Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72(1-2): 248-254.
- Cano, A., Hernández-Ruiz, J., and Arnao, M.B. (2024). Role of exogenous melatonin in plant biotechnology: Physiological and applied aspects. *Crit. Rev. Plant Sci.* 43(6): 395-404.
- Damalas, C.A., and Koutroubas, S.D. (2021). In: Hayat, S., Siddiqui, H., Damalas, C.A. (eds) *Salicylic Acid - A Versatile Plant Growth Regulator*. Springer, pp 65–76.
- Fan, S., Wu, H., Gong, H., and Guo, J. (2022). The salicylic acid mediates selenium-induced tolerance to drought stress in tomato plants. *Sci. Hort.* 300: 111092.
- Ghorbani, A., Pehlivan, N., Zargar, M., and Chen, M. (2024a). Synergistic role of melatonin and hydrogen sulfide in modulating secondary metabolites and metal uptake/sequestration in arsenic-stressed tomato plants. *Sci. Hort.* 331: 113159.
- Ghorbani, A., Pehlivan, N., Zargar, M., and Chen, M. (2024b). Synergistic role of melatonin and hydrogen sulfide in modulating secondary metabolites and metal uptake/sequestration in arsenic-stressed tomato plants. *Sci. Hort.* 331: 113159.

- González-Villagra, J., Reyes-Díaz, M.M., Tighe-Neira, R., Inostroza-Blancheteau, C., Escobar, A.L., and Bravo, L.A. (2022). Salicylic acid improves antioxidant defense system and photosynthetic performance in *Aristotelia chilensis* plants subjected to moderate drought stress. *Plants* 11(5): 639.
- Haghpanah, M., Hashemipetroudi, S., Arzani, A., and Araniti, F. (2024). Drought tolerance in plants: physiological and molecular responses. *Plants* 13(21): 2962.
- Hasan, M.M., Skalicky, M., Jahan, M.S., Hossain, M.N., Anwar, Z., Nie, Z.-F., Alabdallah, N.M., Brestic, M., Hejnak, V., and Fang, X.-W. (2021). Spermine: its emerging role in regulating drought stress responses in plants. *Cells* 10(2): 261.
- Hayat, S., Siddiqui, H., and Damalas, C.A. (2021). *Salicylic acid-a versatile plant growth regulator*. Springer.
- Hosseini, S., Tahmasebi-Sarvestani, Z., Mokhtassi-Bidgoli, A., Keshavarz, H., Kazemi, S., Khalvandi, M., Pirdashti, H., and Nicola, S. (2023). Functional Quality, Antioxidant Capacity and Essential Oil Percentage in Different Mint Species Affected by Salinity Stress. *Chem. Biodiver.* 20(4): e202200247-e202200247.
- Hosseini, S.J., Tahmasebi-Sarvestani, Z., Pirdashti, H., Modarres-Sanavy, S.A.M., Mokhtassi-Bidgoli, A., Hazrati, S., and Nicola, S. (2019). Assessment of salinity indices to identify mint ecotypes using intelligent and regression models. *Int. J. Hortic. Sci. Technol* 7(2): 119-137.
- Hosseini, S.J., Tahmasebi - Sarvestani, Z., Pirdashti, H., Modarres - Sanavy, S.A.M., Mokhtassi - Bidgoli, A., Hazrati, S., and Nicola, S. (2021). Investigation of yield, phytochemical composition, and photosynthetic pigments in different mint ecotypes under salinity stress. *Food Sci. Nutr.* 9(5): 2620-2643.
- Jafari, M., and Shahsavar, A. (2021). The effect of foliar application of melatonin on changes in secondary metabolite contents in two citrus species under drought stress conditions. *Front. Plant Sci.* 12: 692735.
- Karumannil, S., Khan, T.A., Kappachery, S., and Gururani, M.A. (2023). Impact of exogenous melatonin application on photosynthetic machinery under abiotic stress conditions. *Plants* 12(16): 2948.
- Kaur, G., Tak, Y., and Asthir, B. (2022). Salicylic acid: a key signal molecule ameliorating plant stresses. *Cereal Res. Commun.* 50(4): 617-626.
- Keshavarz - Mirzamohammadi, H., Tohidi - Moghadam, H.R., and Hosseini, S.J. (2021). Is there any relationship between agronomic traits, soil properties and essential oil profile of peppermint (*Mentha piperita* L.) treated by fertiliser treatments and irrigation regimes? *Ann. Appl. Biol.* 179(3): 331-344.
- Khalid, M., Rehman, H.M., Ahmed, N., Nawaz, S., Saleem, F., Ahmad, S., Uzair, M., Rana, I.A., Atif, R.M., and Zaman, Q.U. (2022). Using exogenous melatonin, glutathione, proline, and glycine betaine treatments to combat abiotic stresses in crops. *Int. J. Mol. Sci.* 23(21): 12913.
- Khalil, N., Elhady, S.S., Diri, R.M., Fekry, M.I., Bishr, M., Salama, O., and El-Zalabani, S.M. (2022). Salicylic acid spraying affects secondary metabolites and radical scavenging capacity of drought-stressed *Eriocephalus africanus* L. *Agronomy* 12(10): 2278.
- Khalvandi, M., Amerian, M., Pirdashti, H., Keramati, S., and Hosseini, J. (2019). Essential oil of peppermint in symbiotic relationship with *Piriformospora indica* and methyl jasmonate application under saline condition. *Ind Crops Prod* 127: 195-202.
- Khalvandi, M., Siosemardeh, A., Roohi, E., and Keramati, S. (2021). Salicylic acid alleviated the effect of drought stress on photosynthetic characteristics and leaf protein pattern in winter wheat. *Heliyon* 7(1).
- Luck, H. (1971). "Catalase," in *Methods of enzymatic analysis*, ed. H.-U. Bergmeyer. (Germany: Elsevier, 2012).
- Luo, Y., Hu, T., Huo, Y., Wang, L., Zhang, L., and Yan, R. (2023). Effects of exogenous melatonin on chrysanthemum physiological characteristics and photosynthesis under drought stress. *Horticulturae* 9(1): 106.
- Mohammadi-Cheraghabadi, M., Modarres-Sanavy, S.A.M., Sefidkon, F., Rashidi-Monfared, S., and Mokhtassi-Bidgoli, A. (2021). Improving water deficit tolerance of *Salvia officinalis* L. using putrescine. *Sci. Rep.* 11(1): 21997.

- Moustafa-Farag, M., Mahmoud, A., Arnao, M.B., Sheteiwy, M.S., Dafea, M., Soltan, M., Elkelish, A., Hasanuzzaman, M., and Ai, S. (2020). Melatonin-induced water stress tolerance in plants: Recent advances. *Antioxidant* 9(9): 809.
- Murali-Baskaran, R.K., Mooventhan, P., Das, D., Dixit, A., Sharma, K.C., Senthil-Nathan, S., Kaushal, P., and Ghosh, P.K. (2022). The future of plant volatile organic compounds (pVOCs) research: Advances and applications for sustainable agriculture. *Environ. Exp. Bot.* 200: 104912.
- Murch, S.J., and Erland, L.A. (2021). A systematic review of melatonin in plants: an example of evolution of literature. *Front. Plant Sci.* 12: 683047.
- Nakano, Y., and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22(5): 867-880.
- Ostadi, A., Javanmard, A., Amani Machiani, M., Sadeghpour, A., Maggi, F., Nouraein, M., Morshedloo, M.R., Hano, C., and Lorenzo, J.M. (2022). Co-application of TiO<sub>2</sub> nanoparticles and arbuscular mycorrhizal fungi improves essential oil quantity and quality of sage (*Salvia officinalis* L.) in drought stress conditions. *Plants* 11(13): 1659.
- Pan, X., Zhao, Q., and Zhao, T. (2021). Plant growth, antioxidative enzyme, and cadmium tolerance responses to cadmium stress in *Canna orchoides*. *Hortic. Plant J.* 7(3): 256-266.
- Rahimi, A., Mohammadi, M.M., Siavash Moghaddam, S., Heydarzadeh, S., and Gitari, H. (2022). Effects of stress modifier biostimulants on vegetative growth, nutrients, and antioxidants contents of garden thyme (*Thymus vulgaris* L.) under water deficit conditions. *J. Plant Growth Regul.* 41(5): 2059-2072.
- Rajput, S.B., Tonge, M.B., and Karuppayil, S.M. (2014). An overview on traditional uses and pharmacological profile of *Acorus calamus* Linn.(Sweet flag) and other *Acorus* species. *Phytomedicine* 21(3): 268-276.
- Ranjan, A., Sinha, R., Singla - Pareek, S.L., Pareek, A., and Singh, A.K. (2022). Shaping the root system architecture in plants for adaptation to drought stress. *Physiol. Plant.* 174(2): e13651.
- Rehaman, A., Mishra, A.K., Ferdose, A., Per, T.S., Hanief, M., Jan, A.T., and Asgher, M. (2021). Melatonin in plant defense against abiotic stress. *Forests* 12(10): 1404.
- Salvi, P., Manna, M., Kaur, H., Thakur, T., Gandass, N., Bhatt, D., and Muthamilarasan, M. (2021). Phytohormone signaling and crosstalk in regulating drought stress response in plants. *Plant Cell Rep.* 40: 1305-1329.
- Siddiqui, M., Khan, M., Mukherjee, S., Basahi, R., Alamri, S., Al-Amri, A., Alsubaie, Q., Ali, H., Al - Munqedhi, B., and Almohisen, I. (2021). Exogenous melatonin - mediated regulation of K<sup>+</sup>/Na<sup>+</sup> transport, H<sup>+</sup> - ATPase activity and enzymatic antioxidative defence operate through endogenous hydrogen sulphide signalling in NaCl - stressed tomato seedling roots. *Plant Biol.* 23(5): 797-805.
- Talaat, N.B. (2023). Drought stress alleviator melatonin reconfigures water-stressed barley (*Hordeum vulgare* L.) plants' photosynthetic efficiency, antioxidant capacity, and endogenous phytohormone profile. *Int. J. Mol. Sci.* 24(22): 16228.
- Tiwari, R.K., Lal, M.K., Kumar, R., Chourasia, K.N., Naga, K.C., Kumar, D., Das, S.K., and Zinta, G. (2021). Mechanistic insights on melatonin - mediated drought stress mitigation in plants. *Physiol. Plant.* 172(2): 1212-1226.
- Zahra, N., Hafeez, M.B., Kausar, A., Al Zeidi, M., Asekova, S., Siddique, K.H., and Farooq, M. (2023). Plant photosynthetic responses under drought stress: Effects and management. *J. Agron. Crop Sci.* 209(5): 651-672.
- Zulfiqar, F., Chen, J., Finnegan, P.M., Younis, A., Nafees, M., Zorrige, W., and Hamed, K.B. (2021). Application of trehalose and salicylic acid mitigates drought stress in sweet basil and improves plant growth. *Plants* 10(6): 1078.

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# بررسی تاثیر الیستورهای اسید سالیسیلیک و ملاتونین بر فعالیت آنزیم‌های آنتی‌اکسیدان و خصوصیات مورفولوژیک گیاه دارویی *Acorus calamus* تحت تنش خشکی

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Amiri-Maleki, A., Ranjbar, Gh. A., Kazemitabar, S. K., Ghasemi-Omran, V and Hosseini, S.J. (2024). The effects of salicylic acid and melatonin elicitors on the antioxidant enzymes activity and morphological characteristics of *Acorus calamus* under drought stress. *J Plant Mol Breed.* 12 (2): 117-132  
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**چکیده:** گیاهان دارویی به بهبود سلامت انسان کمک می‌کنند، تقویت سیستم ایمنی، کاهش التهاب و درمان بیماری‌ها را تسهیل می‌کنند. برای بررسی تأثیر تنش خشکی و محلول‌پاشی ملاتونین و اسیدسالیسیلیک بر شاخص‌های بیوشیمیایی و مورفولوژیکی گیاه دارویی *Acorus calamus*، آزمایشی انجام شد. نتایج نشان داد که کاربرد الیستورهای ملاتونین و اسید سالیسیلیک موجب افزایش فعالیت آنزیم پراکسیداز در شرایط تنش خشکی ۱۰ و ۲۰ روز پس از قطع آبیاری شد. همچنین اسید سالیسیلیک در تیمار ۱۰ روز پس از تنش موجب افزایش فعالیت آنزیم کاتالاز شد. کاربرد ملاتونین و اسید سالیسیلیک در ۲۰ روز پس از تنش موجب افزایش فعالیت آنزیم کاتالاز شد. در شرایط عدم تنش، کاربرد تیمارهای اسید سالیسیلیک و ملاتونین در ۱۰ روز پس از قطع آبیاری موجب افزایش وزن خشک گیاه در مقایسه با تیمار شاهد شد. کاربرد ملاتونین در شرایط تنش خشکی (۲۰ روز بعد از تنش) موجب افزایش وزن خشک گیاه در مقایسه با تیمار شاهد شد. در نهایت، در حالی که پاسخ‌های گیاه به تنش و محرک‌ها در ویژگی‌های مختلف متفاوت بود، به‌طور کلی می‌توان گفت که اسید سالیسیلیک و ملاتونین تأثیر مثبتی بر ویژگی‌های بیوشیمیایی و مورفولوژیکی گیاه *A. calamus* تحت تنش خشکی داشتند.

**کلمات کلیدی:** شاخص‌های بیوشیمیایی، تنش خشکی، محلول‌پاشی، گیاه دارویی، شاخص‌های مورفولوژیکی.