Journal of Medicinal Plants and By-Products (2025) 1: 54– 62

Reduction of Drought Stress Effects on Saffron (*Crocus sativus* L.) using Phytohormones

Seyed Masoud Ziaei*[1], Abbas Khashei siuki[2,3], Ahmad Ahmadian[2] and Alijan Salarian[2]

[1] *Department of Plant Production, Higher Education Complex of Saravan, Saravan, Iran*
[2] *Department of Plant Production, University of Torbat Heydarieh, Torbat Heydarieh, Iran and Saffron Institute, University of Torbat Heydarieh, Torbat Heydarieh, Iran*
[3] *Department Water Science and Engineering, University of Birjand, Birjand, Iran*

Article Info

Article Type
Original Article

Received: 25 May 2024
Accepted: 03 August 2024

***Corresponding author**
m.ziaei@saravan.ac.ir

ABSTRACT

The effects of global warming and climate changes on plant growth in arid and semiarid regions have prompted the implementation of crop improvement strategies to mitigate these adverse impacts. One of these strategies involves the application of foliar treatments. A study including two irrigation levels with 70% and 50% field capacity, representing mild and severe drought stresses, respectively, and five spraying treatments were conducted. The spraying treatments included no spraying (control), zinc sulfate ($ZnSO_4$: 3/1000), salicylic acid (SA: 40 mg/l), methyl jasmonate (MeJA: 2 mg/l), and auxin (IAA: 1/5 mg/l). It was observed that the levels of chlorophylls a and b in the leaves increased in response to drought stress. Furthermore, an escalation in the severity of drought stress resulted in heightened levels of secondary metabolites (crocin, picrocrocin, and safranal). Conversely, the dry weight of daughter corms decreased due to drought stress. Additionally, the proline, soluble sugars contents, and nitrogen, potassium, and zinc absorption were increased in MeJA and IAA-treated daughter corms under mild and severe drought stress conditions. On the other hand, phosphorous uptake decreased significantly under severe drought stress compared to mild stress conditions. Likewise, the application of MeJA and IAA significantly increased stigma and petal yields, as well as the content of secondary metabolites in saffron. Moreover, MeJA and IAA applications enhanced water use efficiency under both mild and severe drought stress conditions, highlighting their potential to reinforce the non-enzymatic defense system, increase daughter corm dry weight and nutrient uptake, improve saffron quality and quantity, and alleviate the adverse effects of drought stress in saffron plants however, further research is warranted to comprehensively understand these effects.

Keywords: Osmoprotectants, Phytohormone, Secondary metabolites, Water use efficiency

How to cite this paper?
Ziaei, S. M., Khashei Siuki, A., Ahmadian, A., Salarian, A. Reduction of Drought Stress Effects on Saffron (*Crocus sativus* L.) using Phytohormones. Journal of Medicinal plants and By-Products, 2025; (1): 54-62. doi: 10.22034/jmpb.2024.365872.1697

INTRODUCTION

In Iran, despite the challenges of climate change and decreased precipitation impacting crop production, saffron stands out as a plant with low water requirements. Various studies have highlighted that water stress can lead to reduced flower yield and negative effects on daughter corms, ultimately impacting the next year's yield. To mitigate the potential yield reduction in drought stress conditions, it is imperative to employ optimal water management practices for saffron cultivation, as well as consider the use of compounds that can bolster the plant's resilience in harsh environmental conditions. Enhancing daughter corms under low soil water conditions has shown the potential to increase yield compared to scenarios without such plant enhancements [1]. The application of foliar spraying represents an effective method for supplying essential nutrients that may be deficient or not readily available in the growing medium [2]. Drought stress significantly alters the availability of various soil nutrients, leading to an imbalance in nutrient uptake balance by the plants and subsequently reducing access to necessary nutrients [3]. Because of multiple environmental factors, such as high soil pH and organic matter deficiency, in arid areas, the foliar application of Zn and Mn can increase yield and reduce the adverse effects of environmental stresses, particularly drought [4]. Phytohormones which are plant-produced molecules present in minute concentrations, play a pivotal role in biochemical communications and the regulation of diverse signaling pathways in response to environmental stress, thereby modulating intra-plant relationships [3]. The major phytohormones include auxin, cytokinin, gibberellin, ethylene, abscisic acid (ABA), brassinosteroids, salicylic acid (SA), jasmonates, and strigolactones, which possess crucial impacts on growth and development and mitigation of environmental stress effects in plants. Likewise, mineral compounds are integral to maintaining cell homeostasis and alleviating the environmental stress impacts on plants, as they are essential for plant nutrition and growth [5]. On the other hand, climatic changes due to high temperatures and low soil moisture reduce the yields of plants in such areas. These substances can alleviate environmental stress effects, such as drought, activate stress-resistance genes, increase antioxidant enzyme activity, create osmoprotectants in cells, synthesize heat shock proteins (HSP) and other stress-resistance proteins, reduce reactive oxygen species (ROS) activity, stabilize the cell membrane, repair DNA, increase leaf chlorophyll content, and reduce heavy metal uptake in plants [6]. Among biotic stresses, high temperatures, and drought can affect phytohormone production, nutrient uptake, stomatal conductance, transpiration rate, photosynthetic activity, enzymatic and non-enzymatic activities, and free radical production. In drought stress conditions, osmoregulators, soluble proteins, proline, soluble sugars, etc. are produced in plants. Drought affects the hormonal balance of auxin and ABA content in plants. Rapid ABA accumulation has been observed in drought and salinity stress conditions [7]. In drought conditions, elevated ABA and SA levels decreased gibberellin and auxin concentrations, and changes in cytokinin levels of roots and shoots have been reported in plants. ABA and ethylene reportedly reduced gas exchange and the contents of chlorophylls a and b in cotton. Drought stress can activate the phytohormones that cause better gene expression and protein synthesis [8]. Exogenous phytohormone application as foliar application is a good method in environmental stress conditions and alleviates stress effects [9, 10]. Insufficient information exists regarding the mechanism of MeJA in ameliorating drought stress damage in saffron. This study aims to examine the impact of foliar application of phytohormones in mitigating oxidative damage caused by drought stress in saffron. Additionally, the study seeks to elucidate the agro-biochemical mechanisms of MeJA in regulating plant responses to drought stress.

MATERIALS AND METHODS

Site Description

The current on-field experiment was carried out for two consecutive years, 2022-2023, at the Torbat Heydarieh Saffron Research Institute farm, situated in the northeast of Iran (34° 17′ N, 59° 12′ E, and 1450 m altitude) with semiarid climate. The average annual temperature and the mean total are 14 °C and 210 mm, respectively. The most significant climatic parameters of the experimental site are shown in Table 1.

Experimental setup and preparation of treatments

This split-plot experiment was conducted as a randomized complete block design with three replicates. The irrigation water was used as the main plot at two levels of 70% FC as the control and 50% FC as the drought stress (After the first irrigation in the first year) treatment and Five spraying treatments, namely no spraying (control), and spraying with zinc sulfate (ZnSO4: 3/1000), salicylic acid (SA: 40 mg/l), methyl jasmonate (MeJA: 2 mg/l), and auxin (IAA: 1/5 mg/l).

Table 1 Monthly rainfall and average temperature during both years of the experiment.

Year	Months	Average temperature (̊C)	Rainfall (mm)
2022	October	16.80	3.00
2022	November	10.70	6.15
2022	December	3.45	2.30
2023	January	-0.60	12.05
2023	February	5.07	40.83
2023	March	12.92	23.62
2023	April	17.03	0.01
2023	May	21.42	15.07
2023	June	27.24	3.30
2023	July	28.00	0.00
2023	August	25.75	0.20
2023	September	21.74	0.00
2023	October	16.82	3.02
2023	November	11.69	11.04

Preparation of Treatments, Field Experiments, and Agronomic Practices

The maternal corm with a uniform weight of approximately 10 grams was sourced from large-scale saffron farmers operating under similar agronomic and ecological circumstances as our research site. Initially, six soil samples were randomly collected from the farm at a depth of 0-30 cm. These samples were promptly transported to the laboratory for the assessment of soil physicochemical properties, as detailed in Table 2.

The experimental land was prepared based on local agricultural practices for saffron. Each plot was prepared at a dimension of 1.5 × 2 m2, which contained six rows of /plot. The distance between rows was 25 cm. One path with the length of one and two (m) was placed between the main and sub-plots, respectively. Treated corms were planted in an equidistance pattern at a depth of 20 cm and intervals of 4 cm on 16 September 2022. Neither pesticide nor herbicide was utilized during the whole growing season. Initial watering, equivalent to 600 m^{-3}/ha, was applied uniformly upon planting, followed by subsequent irrigations based on the farm's field capacity at 50% (2500 m^3/ha) and 70% (3800 m^3/ha) by the volume method. The irrigation method employed was flooding consistently throughout the duration of the experiment. The depth of the irrigation water was determined to be 25 cm using the formula outlined in Eq. (1) [11].

$$d = \rho b \times w \times D / 100 \qquad (1)$$

where d is the height of irrigation water (cm), ρb is soil bulk density (g cm^{-3}), w is the rate of soil moisture content change (%), and D is root depth (25 cm). Soil samples were collected every other day from the experimental plots, and their water content was determined using the gravimetric method, which involves calculating the difference in weight between wet and oven-dried soil samples.

Table 2 Physical and chemical criteria of soil (0–30 cm).

Texture	Available potassium (Ppm)	Available Phosphorous (ppm)	Total nitrogen (%)	Organic carbon, (%)	Electrical conductivity (ds/m)	pH	Sodium, (meq/l)	Calcium+ magnesium (meq/l)
Loamy	448	28	0.04	0.312	5.29	8.39	47.86	14.8

Subsequently, the volume of water required to reach field capacity (FC) was computed by multiplying the plot area by the height of the water. Foliar spraying was conducted twice, with a two-week interval, at the onset of March 2023.

Sampling

Eventually, the saffron flowers were picked up manually. In the second year, the flowers were harvested daily on the mid-morning of 13 November to 28 November 2023 and then counted and weighed daily. Afterward, all stigmas were carefully plucked from the flowers and weighed individually measured. Daughter corms were also lifted from the soil at the end of the first growing season (10 May 2023).

Analytical Methods

Qualitative Traits

The quality of the first and second year saffron samples was determined by measuring the strength of color, flavor, and aroma expressed as crocin, picrocrocin, and safranal, respectively. Based on the ISO/TS 3632–2 (2003) [12] standard method, ~500 mg of dried stigma was transferred to a 1000 ml volumetric flask, and the volume reached 1000 ml by adding 900 ml of distilled water. It was then stirred on a magnetic stirrer (HPMA 700) at 600 rpm for one hour in the dark to mix the solution completely. Afterward, the required deionized water was added to obtain our standard solution at a volume of 1000 ml. In the next step, 20 ml of the solution was poured into a 200 ml Erlenmeyer flask using a pipette, and then distilled water was added until the target line. The solution was remixed thoroughly and passed through silicate filter paper in the dark to remove impurities and obtain a uniform solution. The solution light absorbance was read at 257, 330, and 440 nm for picrocrocin, safranal, and crocin, respectively, using a spectrophotometer. The spectrophotometer readings were converted to the mentioned compounds according to Eq. 2 [12]:

$$A_{1cm}^{1\%}\,(\lambda_{max}) = \frac{D \times 10000}{m \times (100 - H)} \qquad (2)$$

Here, A is light absorption for each qualitative compound, D is the number of readings by the spectrophotometer, m is the dry weight of stigma (g), and H (6%) is the moisture content of the samples.

Biochemical Evaluation

Evaluation of Proline Content

The free proline in leaf samples was quantified, and the absorbance of extracts was measured at 520 nm [13].

Evaluation of Soluble Sugar

Soluble sugar is extremely sensitive to abiotic and biotic stressors impacting crop growth and development and also has a key role in stress pathway signaling [14]. The content of soluble sugar in the cell sap of leaf tissues was determined (3 April) using the phenol sulfuric acid method [15], in which glucose was used as a standard.

Determining the Contents of Nitrogen, Phosphorus, Potassium, and Zinc in Corm Samples

Corm samples were rinsed three times with distilled water, dried in an oven at 70 ˚C for 24 h, and then ground and passed through a 40-mesh sieve. The micro-Kjeldahl method, flame photometry, and spectrophotometry were used to measure total N, K, and P contents, respectively [16, 17, 18]. Zn concentration was determined using an atomic absorption spectrometer (Lambda 365, PerkinElmer, Waltham, MA, US) [19].

Evaluating Water Use Efficiency (WUE)

The productivity of irrigation water was calculated using Eq. 6 based on the yield of saffron stigmas [1].

WUE = Stigma (g)/Irrigation (m^3)

Statistical Analysis

The variance among all the data was analyzed using SAS (*Ver* 9.4) software. Means were compared by Tukey's HSD test ($P \leq 0.05$).

RESULTS

Petal and Stigma Dry Weights

The drought × spraying interaction effect significantly influenced petal dry weight (Table 3). This trait was maximal under mild drought stress conditions in the MeJA spraying treatment, which showed about 33% and 30% increases compared to control and ZnSO4spraying treatments, respectively. At this drought stress level, MeJA and IAA spraying treatments were not significantly different in the petal yield. Similarly, no significant difference was observed between SA and IAA spraying treatments at this drought stress level. However, petal yield was about 8% higher in IAA vs. SA spraying treatments (Table 3). At the severe drought stress level, petal yield was maximal in the MeJA spraying treatment and was 3.5 times greater than the control. Drought stress-induced reduction was minimal in MeJA and IAA spraying treatments, with about a 43% decreased yield compared to mild stress conditions. The utmost drought stress-induced reduction belonged to the control treatment, in which petal yield declined by about 4 times compared to mild stress. After the MeJA spraying treatment, the highest petal yields were respectively measured in IAA, SA, ZnSO4, and control treatments. The highest stigma yield was achieved with MeJA and IAA spraying treatments under mild drought stress conditions, resulting in a three times increase compared to the control treatment. SA and $ZnSO_4$ spraying treatments were not statistically different. The control and ZnSO4spraying treatments did not differ at this stress level, but $ZnSO_4$ spraying produced a greater stigma yield. Under severe drought stress conditions, the stigma yield was higher (about 10 times) in the MeJA spraying treatment than in the control (Table 3). MeJA and auxin spraying treatments each produced 4 times stigma yields relative to SA and ZnSO4spraying treatments under severe drought stress conditions. The minimum stigma yield (a 5-fold reduction) was observed in the control treatment under severe vs. mild drought stress conditions while a 27% reduction occurred in the MeJA spraying treatment. This study scrutinized the potential application of growth regulators (auxin, MeJA, and SA) because of their ability to improve the morpho-physiological growth and biochemical properties of saffron. In this research, saffron quantity and quality were affected by drought treatments, while drought effects on saffron plant growth were adjusted by MeJA and SA application. As a major disturbing factor in growth and photosynthetic/metabolic activities, drought can reduce saffron yield. MeJA and SA are known as plant growth stimulators and drought effect moderators that increase root growth and maintain the balance of metabolic activities in plants [20]. IAA is known to play a role in growth and development, flowering stimulation, and ripening in plants. Evidence indicates that MeJA and SA application mitigates drought stress-induced impacts [21].

Leaf Proline Content, total Soluble Sugar and Photosynthesis Pigments (Osmoprotectants)

The maximum proline content was obtained in the MeJA spraying treatment, which was about 90% more than the control treatment (Table 4). The greatest proline content was found with MeJA spraying under severe drought stress, with about a 60% rise compared to mild drought stress conditions. No significant differences were observed between SA, $ZnSO_4$, MeJA, and IAA treatments under severe drought stress. However, MeJA and IAA treatments contained higher proline contents. In severe drought stress, MeJA spraying resulted in about 25% more proline content than the control conditions (Table 4). The proline content in the leaves doubled more than twice in the control treatment as drought stress increased from mild to severe. This represented the highest level of proline biosynthesis compared to mild stress conditions in the spraying treatments. Soluble sugar content was significantly influenced by the drought × spraying interaction effect.

This trait was uppermost in the MeJA + IAA spraying treatment, with a 34% increase compared to the control (Table 4). Soluble sugars were generally augmented by 20% in the MeJA spraying treatment with a rising drought stress level, compared to a 31% elevation in the control treatment with an increase in the drought stress level. Soluble sugars were maximized in the MeJA spraying treatment under severe stress conditions, however, this treatment and IAA spraying were not extremely distinct at this drought level. IAA spraying was not significantly different from SA, $ZnSO_4$, and control treatments in terms of soluble sugar content. Nevertheless, the SA treatment contained a higher content of soluble sugars. Chlorophyll content was significantly affected by drought stress and increased by about 50% with increasing drought severity from mild to severe stress. The spraying treatments were significantly different at the 5% level (Table 5). The highest chlorophyll content, with about a 30% increase versus the control, was measured in the MeJA spraying treatment. The other spraying treatments were allocated to a single statistical group. Chlorophyll b content was significantly different in irrigation treatments and showed a two-fold elevation by drought stress intensification. No statistically significant differences were observed between the spraying treatments, and spraying seemingly did not significantly affect changes in chlorophyll b content. Nonetheless, the $ZnSO_4$ treatment contained the utmost chlorophyll b content. Carotenoid content rose to 30% by intensifying drought stress from mild to severe (Table 5). MeJA application can reportedly alleviate the adverse effects of drought stress by increasing plant height, biomass, photosynthetic pigments, and non-enzymatic defense system activities [22]. Under drought stress conditions, MeJA and SA spraying induced biochemical, morphological, and physiological responses and enhanced drought tolerance in chamomile [23]. The studied traits were yield, quality, element uptake, photosynthetic pigments, proline content, and soluble sugars. JA could increase chlorophyll and carotenoid contents, and MeJA augmented maize biomass under drought stress conditions compared to control settings. Exogenous MeJA application led to increases in leaf proline content and soluble sugars under drought stress conditions, maintained cell osmotic potential, and osmosis regulation; these compounds also reduce ROS activity. MeJA application elevated leaf proline content under drought stress conditions compared to control settings [24], which corresponds to our results. Drought stress caused starch decomposition and sugar accumulation in the plant. A significant correlation between soluble sugar accumulation and drought stress tolerance [25]. MeJA application amplified proline and sugar contents in the soy plant, which agrees with the results of this study.

ABA hormone biosynthesis and, consequently, drought tolerance in plants are improved with MeJA application. ABA hormone rose in soybean and oat plants under drought stress conditions. The flowering rate, flower yield, and stigma yield of saffron were improved due to an increase in produced assimilates and their transfer to corms and underground organs, which corresponds to our results [26].

Dry Weight of Daughter Corm and Concentrations of Ions (N, P, K^+, Zn)

Daughter corm dry weight was significantly affected by drought effects and declined by 32% along with intensifying drought stress from mild to severe (Table 7). Among the spraying treatments, the greatest daughter corm dry weight was recorded in the MeJA treatment, which was 18% more than the control treatment. This trait was not significantly different between $ZnSO_4$ and IAA spraying treatments, but a higher daughter corm dry weight was obtained by IAA spraying. After the MeJA spraying treatment, the highest daughter corm dry weights were respectively recorded in IAA, SA, $ZnSO_4$, and control treatments (Table 7).

The interaction effect of drought and spraying significantly affected corm N content (Table 6). N uptake was not significantly different among spraying treatments at the mild drought stress level, but the highest N uptake under severe drought stress belonged to the MeJA + IAA treatment. The lowest N content was recorded in the control treatment, with 34% less than the MeJA treatment. In all spraying treatments, N uptake was generally higher in mild vs. severe stress conditions. N uptake was lower in the control treatment under mild compared to severe stress conditions. The interaction effect of drought and spraying was significant on K uptake, which was uppermost under mild drought stress conditions with MeJA spraying (Table 6). The least K uptake belonged to the control treatment under mild drought stress conditions, which was about 14% lower than the MeJA spraying treatment. K uptake decreased in all spraying treatments under severe vs. mild stress conditions. K uptake was significantly influenced by drought stress, and the most K uptake reduction (22%) belonged to the MeJA treatment in mild stress conditions. The same treatment showed the highest K uptake under severe stress conditions compared to the other treatments. In these soil moisture conditions, no statistically significant differences were observed among MeJA, IAA, and SA treatments. The least K uptake was measured under severe stress conditions in $ZnSO_4$ spraying and control treatments, which was 13% lower than that in the MeJA spraying treatment. Zn uptake was significantly affected by the drought × spraying interaction effect. Under mild stress conditions, Zn uptake was maximal in the $ZnSO_4$ spraying treatment, with a 35% elevation compared to the control treatment. Zn uptake decreased in all spraying treatments along with drought stress intensification from mild to severe. The utmost reduction caused by drought stress intensification belonged to the control treatment, with a 26% lower uptake than mild stress conditions. A drought-induced Zn reduction of about 16% was recorded in the $ZnSO_4$ spraying treatment, which showed a lower decrease than the other spraying treatments (Table 6). Sulfur has been shown to regulate plant's metabolic processes and elevate tolerance to environmental stresses. Microelements (e.g., Zn) amplify resistance to environmental stresses and redouble crop yield through glucose metabolism and transportation, membrane stability maintenance, and enzymatic system activation in plants [27]. Such elements as Zn and N play an essential role in cell membrane permeability. Similar to phytohormones, plant nutrients can reduce the adverse effects of abiotic stresses. Observations indicate that macroelements (e.g., N) can increase plant photosynthesis in response to multiple abiotic stresses [28]. P can augment production and a strong root system [29]. Micronutrients can regulate cell activity and reduce abiotic stresses by activating several enzymes. Some plant growth regulators, including SA, gibberellins, auxins, cytokinins, and ABA, have reacted to drought [30].

Phytohormones regulate internal and external stimulators as well as signaling pathways, in addition to stress responses. Auxin application effectively contributes to drought stress management in plants, which corresponds to our results. Auxin concentration largely influences the growth of lateral buds, and its increase reduces lateral bud growth in plants (apical dominance). Increased apical bud growth and lateral bud dormancy at high auxin concentrations in apical buds depend on their sensitivity to auxin concentration. Lateral buds are more sensitive to auxin than apical buds. Thus, the decreased number and increased weight of daughter corms can explain daughter corm enlargement in the presence of auxin [31], as evidenced by our experimental results. Elsewhere, elevated auxin concentrations could increase growth indices and, consequently, daughter corm weight in saffron, which further supports the increased daughter corm weight in this study.

Water Use Efficiency (WUE)

WUE was significantly influenced by the drought × spraying interaction effect. This trait was utmost under mild drought stress conditions in the MeJA and auxin spraying treatments. Despite a greater WUE in the MeJA treatment, the two treatments did not differ significantly (Fig. 1).

Table 3 Petal and stigma dry weight (kg/ha) of saffron primed with various spraying agents and grown under drought stress on the farm during season 2022-2023.

Treatments	Petal dry weight	Stigma dry weight
W0×S1	30.933 b	2.480 bc
W0×S2	35.314 a	7.146 a
W0×S3	23.516 c	2.180 c
W0×S4	25.912 c	2.446 bc
W0×S5	35.117 ab	6.932 a
W1×S1	13.250c	0.983 bc
W1×S2	22.351 a	4.130 a
W1×S3	6.358 e	0.413 c
W1×S4	9.452 d	0.880 c
W1×S5	19.919 b	3.813 a
W	**	**
P	**	**
W×P	ns	**

Table 4 The content (mg/g Fw) of proline and total soluble sugar of saffron primed with various priming agents and grown on the farm under drought stress in year of 2023.

Treatments	Soluble sugar	Proline
W × S		
W0×S1	9.54 b	35.30 ab
W0×S2	10.89 a	40.68 a
W0×S3	7.69 c	22.02 c
W0×S4	9.49 b	29.47 b
W0×S5	10.38 ab	36.05 ab
W1×S1	11.34 b	56.40 a
W1×S2	12.79 a	69.93 a
W1×S3	10.91 b	50.15 a
W1×S4	10.98 b	54.78 a
W1×S5	12.12 ab	65.51 a
W	**	**
P	**	ns
W×P	**	**

Table 5 The content (mg/g Fw) of leaf chlorophyll (Chl), Chl a, Chl b and Carotenoieds of saffron with various spraying agents and grown on the farm under water stress in year of 2023.

Treatments	Chl a	Chl b	Carotenoids
Drought stress (W)			
W0	0.83 b	8.22 b	0.51 b
W1	1.25 a	18.94 a	0.70 a
Spraying (S)			
S1	1.06 a	13.72 a	0.60 a
S2	1.14 a	14.29 a	0.62 a
S3	0.86 b	13.05 a	0.54 a
S4	1.04 a	13.42 a	0.58 a
S5	1.10 a	13.88 a	0.62 a
W	**	**	**
S	*	ns	ns
W×S	ns	ns	ns

At this drought stress level, WUE was about 3 times higher in the MeJA and auxin spraying treatments than in the other treatments. SA and $ZnSO_4$ spraying treatments and the control were not different significantly at both drought levels. Likewise, the MeJA and auxin spraying treatments yielded the highest WUE vs. the control (> 8 times) under severe drought stress. The most reduction in WUE (about 3 times) was measured in the control treatment at both drought levels.

Table 6 The amounts of nitrogen (N), phosphorous (P), potassium (K+), zinc (Zn) and Dry weight of daughter corm in corm tissues of saffron with various spraying agents and grown on the farm under drought stress in year of 2023.

Treatments	N (%)	P (%)	K+ (%)	Zn (ppm)
W0×S1	3.22 a	0.35 a	0.91 c	28.33 c
W0×S2	3.35 a	0.44 a	1.12 a	31.83b
W0×S3	3.04 a	1.48 a	0.88 d	22.65 d
W0×S4	3.19 a	0.39 a	0.89 d	34.96 a
W0×S5	3.43 a	0.38 a	0.96 b	31.33 b
W1×S1	2.46 b	0.27 a	0.77 a	23.20 b
W1×S2	3.16 a	0.28 a	0.79 a	25.96 b
W1×S3	2.01 c	0.35 a	0.68 b	15.40 c
W1×S4	2.09 c	0.29 a	0.69 b	30.90 a
W1×S5	2.90 a	0.27 a	0.78 a	23.36 b
W	**	*	**	**
P	*	ns	**	**
W×P	*	ns	**	*

Table 7 Dry weight of daughter corm in corm tissues of saffron and amounts phosphorous (P) with various spraying agents and grown on the farm under drought stress in year of 2023.

Treatments	Dry weight of daughter corm (g/plant)	P (%)
W0	5.00 a	0.73 a
W1	3.40 b	0.42 b
S1	4.16 c	0.58 a
S2	4.71 a	0.60 a
S3	3.86 e	0.57 a
S4	4.01 d	0.59 a
S5	4.38 b	0.57 a
W	**	*
S	**	ns
W×S	ns	ns

Table 8 The amounts (absorption/nm) of crocin, picrocrocin, and safranal in stigmas of saffron with various spraying agents and grown on the farm under drought stress in year of 2023.

Treatments	Crocin	Picrocrocin	Safranal
W0	115.96 b	68.47 b	35.81 b
W1	121.79 a	72.64 a	38.69 a
S1	119.28 ab	70.65 bc	37.43 b
S2	122.99 a	73.53 a	39.74 a
S3	115.54 b	67.98 d	33.27 c
S4	114.63 b	70.10 c	37.01 b
S5	121.94 a	71.19 b	38.78 ab
W	**	**	**
S	*	**	**
S×P	ns	ns	ns

Data are represented as mean with three replicates. Different letters represent statistically significant differences with Tukey's HSD ($P \leq 0.05$). Statistical differences at $P \leq 0.05$ and $P \leq 0.01$ shown with * and **, respectively, while NS means non-significant. W0 and W1 are irrigation at 70% and 50% FC, respectively. S1, S2, S3, S4 and P5 are spraying with SA, MeJA, Non-spraying (Control), ZnSo4 and IAA, respectively.

WUE generally declined in all treatments with the increased drought stress level, but the most decline occurred in the control treatment (Fig. 1). Indole-3-acetic acid (IAA) is the most abundant plant hormone of the auxin class that is mainly synthesized from the amino acid tryptophan (Trp). IAA activates other hormones involved in stress and ROS production [32]. In rice crops, auxin application resulted in good pollen tube growth and, ultimately, appropriate pollination and fertilization at high temperatures. In wheat, the estimation of exogenous auxin application under thermal stress conditions revealed that using IAA (1 µm) could produce a higher number of seeds and increase yield by 6-8%. JA raises water uptake by plants, and MeJA increases the accumulation of osmoprotectants and adaptable elements to elevate chlorophyll content, antioxidant activity, and leaf gas exchange for stomata closure and WUE improvement [33].

Contents of Crocin, Picrocrocin, and Safranal (Secondary Metabolites)

Secondary metabolites increase due to drought. The active ingredient increased by about 5% for crocin and picrocrocin and 7% for safranal under mild drought stress (Table 8). A 6% more crocin concentration was obtained in the MeJA and auxin spraying treatments than in the control. The other spraying treatments did not differ significantly from the control. Picrocrocin was uppermost in spraying with MeJA, followed by auxin, SA, $ZnSO_4$, and control treatments, respectively. MeJA and auxin caused about a 5% elevation compared to the control. Safranal concentration was not significantly different between SA and $ZnSO_4$ treatments (Table 8). JA activates internal phytohormones and polyamines [34]. Evidence shows that JA improves enzymatic and non-enzymatic defense systems in plants. Changes caused by MeJA and auxin spraying could probably elevate the contents of soluble sugars, proline, and secondary metabolites (crocin, picrocrocin, and auxin) in the present study, which agrees with previous studies. In a study, SA use (1 mM) led to crocin content elevation and stronger antioxidant activity in stigmas among different treatments, with no negative effect on safranal content [35]. Similarly, the production of soluble carbohydrates, sugars, and secondary metabolites increased with SA application [36].

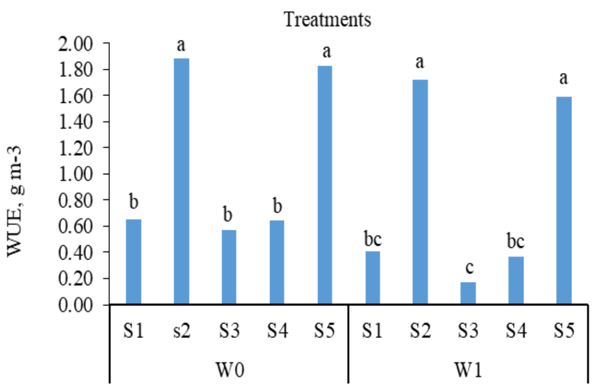

Fig. 1 Water use efficiency (WUE) of saffron with various spraying agents and grown on the farm under drought stress during 2023. Data are represented as mean with three replicates. Different letters represent statistically significant differences with Tukey's HSD (P≤0.05). W0 and W1 are irrigation at 70% and 50% FC, respectively. S1, S2, S3, S4 and P5 are spraying with SA, MeJA, Non-spraying (Control), ZnSo4 and IAA, respectively.

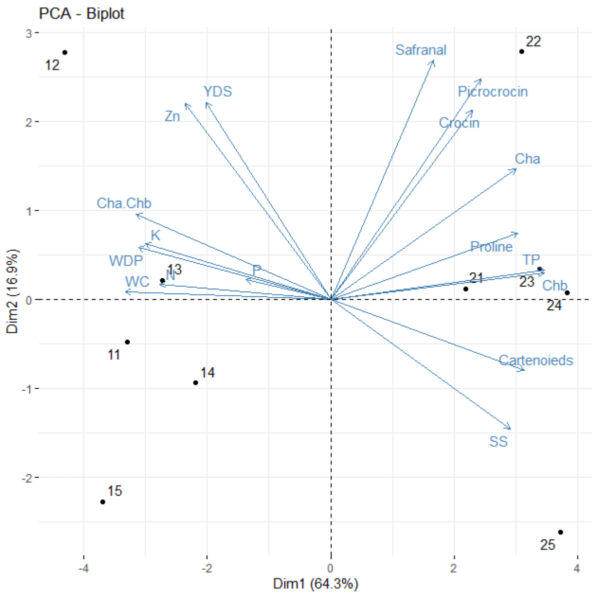

Fig. 2 PCA biplot graphics measured variable in saffron grown under different spraying. 11= Irrigation at 70% FC and spraying with SA, 12= Irrigation at 70% FC and Spraying with MeJA, 13= Irrigation at 70% FC and spraying with Non-spraying (Control), 14=Irrigation at 70% FC and spraying with ZnSo4, 15= Irrigation at 70% FC and spraying with IAA, 21= Irrigation at 50% FC and spraying with SA, 22= Irrigation at 50% FC and Spraying with MeJA, 23= Irrigation at 50% FC and spraying with Non-spraying (Control), 24= Irrigation at 50% FC and spraying with ZnSo4, 25= Irrigation at 50% FC and spraying with IAA.

Fig. 3 Spraying with IAA (below) and control (a bow).

JA and its derivatives are complex compounds affecting a wide range of physiological and developmental reactions in plants [37]. JA is a compound that prevents leaf aging and fall, reduces free radical activity, stimulates ethylene biosynthesis, and enhances the enzymatic defense system in plants [37].

Principal Component Analysis (PCA) of Variables

According to Figure 2, the most positive effect on the yield of saffron was observed in the treatment of mild drought stress (70 % FC) and spraying with MeJA, while in the same treatment, in severe drought stress (50% FC) conditions, qualitative traits such as crocin, picrocrocin, safranal, chlorophyll a, and proline had the most positive effect. Therefore, it shows that the quality of saffron increased in dry conditions. These two treatments have the most positive impact on the yield and quality of saffron flowers (Fig 2).

CONCLUSIONS

Drought stress effects were reduced by MeJA and auxin spraying compared to control conditions. Photosynthetic pigments, osmoprotectants, the non-enzymatic defense system, and element uptake were all improved with MeJA and auxin application, thereby increasing daughter corm weight in saffron plants. Due to the mentioned improved conditions, flowering in this plant was enhanced by spraying under drought stress conditions as opposed to the control treatment. Therefore, spraying seems to boost saffron quantity and quality under drought stress conditions.

Author Contribution Statement

All authors contributed to writing and reviewing the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

This research has been financially supported by the Saffron Institute, University of Torbat Heydarieh. The grant number was 155092.

Declarations

Conflict of Interest

The authors have no conflict of interest to declare.

REFERENCES

1. Aghhavani Shajari M., Rezvani Moghaddam P., Ghorbani R., Koocheki A.R. Does nutrient and irrigation managements alter the quality and yield of saffron (*Crocus sativus* L.). Agric. Water Manag. 2022; 267: 107629.
2. Cakmak I. Role of zinc in protecting plant cells from reactive oxygen species. New Phytologist Trust. 2008; 146, 185–205.
3. Shabir H.W., Vinay K., Varsha S., Saroj K.S. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J. 2016; 4 (3), 162.
4. Khan M., Qasim M., Jamil M. Effect of different levels of Zinc on the extractable Zinc content of soil and chemical composition of rice. Asian J. Plant Sci. 2002; 1: 20-21.
5. Kazan K. Diverse roles of jasmonates and ethylene in abiotic stress tolerance. Trends Plant Sci. 2015; 20 (4), 219–229.
6. Diouf I.A., Derivot L., Bitton F., Pascual L., Causse M. Water deficit and salinity stress reveal many specific QTL for plant growth and fruit quality traits in tomato. Front. Plant Sci. 2018; 6 (9), 279.
7. Hussain H.A., Hussain S., Khaliq A., Ashraf U., Anjum S.A., Men S. Chilling and drought stresses in crop plants: Implications, cross talk, and potential management opportunities. Front. Plant Sci. 2018; (9). 193-202/
8. Kosakivska I.V., Vasyuk V.A., Voytenko L.V. The effects of moderate soil drought on phytohormonal balance of triticum aestivum l. *triticum spelta* l. Cereal Res. Commun. 2002; (7). 291-304.
9. Pandey D., Goswami C., Kumar B. Physiological effects of plant hormones in cotton under drought. Biol. Plantarum. 2003; (47). 535–540.
10. Alscher R.G., N. Erturk L.S. Heath. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J. Exp. Bot. 2002; 53 (372). 1331-1341
11. Ziaei S.M., Nezami A., khazaie H.R. Evaluation of different levels of soil moisture and seed hydro perimination on yield and yield components of *Vigna radiate* L. in Mashhad climate. J. Plant Ecophysiol. 2020; 12(42): 91-103.
12. Iranian National of Saffron Standard. Institute of Standard and Industrial Research of Iran. Karaj. 2003.
13. Bates I.S. Waldern R.P., Teare I.D. Rapid determination of free proline for water stress studies. Plant Soil 1973; 39: 205-207.
14. Rosa M., Prado C., Podazza G., Interdonato R., González J.A., Hilal M. Prado F.E. Soluble sugars: Metabolism, sensing and abiotic stress: A complex network in the life of plants. Plant Sign. Behav. 2009; 4(5): 388-393.
15. Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A., Smith F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 1956; 28(3): 350-356.
16. Bremner J.M., Mulvaney C.S. Nitrogen-Total. In: A.L. Page ed, Methods of soil analysis, part 2, American Society of Agronomy, Madison, Wisconsin. 1982; 591–622.
17. Dadkhah A., Amini dahghi M., Kafi M. Effect of different levels of nitrogen and phosphorus fertilizers on quantitative and qualitative yield of German chamomile. Iranian J. Field Crops Res. 2012; 10(2), 321-326.
18. Olsen S.R., Sommers L.E. Phosphorus. In: Page A.L. ed. Methods of soil analysis, part 2, Chemical and Microbiological properties. Soil Sci. Soc. Am. J. Madison. 1982; 403-430.
19. Ebdon, L. An Introduction to Atomic Adsorption Spectroscopy. Publication Information: London: Heyden, 1982.
20. Cenzano A., Vigliocco A., Kraus T., Abdala G. Exogenously applied jasmonic acid induces changes in apical meristem morphology of potato stolons. Ann. Bot. 2003; 91: 915–919
21. Tavallali V., Karimi S. Methyl jasmonate enhances salt tolerance of almond rootstocks by regulating endogenous phytohormones, antioxidants activity and gas-exchange. J Plant Physiol. 2019; 234–235: 98–105
22. Hasanuzzaman M., Nahar K., Bhuiyan T.F., Anee T.I., Inafuku M., Oku H., *et al*. Salicylic acid: an all-rounder in regulating abiotic stress responses in plants. In: Phytohormones-signaling mechanisms and crosstalk in plant development and stress responses. El-Esawi, M.A., Intech Open. 2017.

23. Nazrli H., Ahmadi A., Hadian J. Salicylic acid and methyl jasmonate enhance drought tolerance in chamomile plants. J Herb Med. Pharmacol. 2014; 3: 87–92.
24. Chen J., Yan Z., Li X. Effect of methyl jasmonate on cadmium uptake and antioxidative capacity in Kandelia obovate seedlings under cadmium stress. Ecotoxicol. Environ. Saf. 2014; 104: 349–356.
25. Anjum S.A., Tanveer M., Hussain S., Tung S.A., Samad R.A., Wang L., *et al*. Exogenously applied methyl jasmonate improves the drought tolerance in wheat imposed at early and late developmental stages. Acta Physiol. Planta. 2016; 38(1): 25
26. Mollafilabi A.S. Khorramdel. Effects of cow manure and foliar spraying on agronomic criteria and yield of saffron (*Crocus sativus* L.) in a six-year-old farm. Saffron Agron. Tech. 2016;3: 237-249.
27. Michael L.C.Y., Espada-Y Gil F., Fuentes Ortiz G., Santamaria J.M., Gonzalez Mendoza D. Bioaccumulation and changes in the photosynthetic apparatus of *Prosopis juliflora* exposed to copper. Bot. Sci. 2016; 94(2): 323–330.
28. Venugopalan V.K., Nath R., Sengupta K., Nalia A., Banerjee S., Sarath Chandran M.A., *et al*. The response of lentil (*Lens culinaris* Medik.) to soil moisture and heat stress under different dates of sowing and foliar application of micronutrients. Front. Plant Sci. 2021; 10, 679469.
29. Ramireddy E., Hosseini S.A., Eggert K., Gillandt S., Gnad H., von Wirén N. Root engineering in barley: Increasing cytokinin degradation produces a larger root system, mineral enrichment in the shoot and improved drought tolerance. Plant Physiol. 2018; 177, 1078–1095.
30. Chen K., Wang Y., Zhang R., Zhang H., Gao C. CRISPR/Cas genome editing and precision plant breeding in agriculture. Annu. Rev. Plant Biol. 2019; 70, 667–697.
31. Sanchez-Romera B. Regulation of root hydraulic properties by methyl jasmonate, reactive nitrogen species and arbuscular mycorrhizae. 2014; Vol. 180 (Granada: Universidad de Granada)
32. Xiong L., Gong Z., Rock C.D., Subramanian S., Guo Y., Xu W. Modulation of abscisic acid signal transduction and biosynthesis by an Sm-like protein in arabidopsis. Dev. Cell 2001; 1, 771–781.
33. Nagarathna T.K., Shadakshari Y.G., Jagadish K.S. Sanjay M.T. Interactions of auxin and cytokinins in regulating axillary bud formation in sunflower (*Helianthus annuus* L.). Helia 2010; 33(52): 85-94.
34. Tajik S. Zarinkamar F. Niknam, V. Effects of salicylic acid on carotenoids and antioxidant activity of Saffron (*Crocus sativus* L.). Appl. Food Biotechnol. 2015; 2(4): 33-37
35. Ghasemzadeh A, Jaafar H, Karimi E, Ibrahim M. Involvement of salicylic acid on antioxidant and anticancer properties, anthocyanin production and chalcone synthase activity in ginger (*Zingiber officinale* roscoe) varieties. Int. J. Mol. Sci. 2012; 13: 14828-14844
36. Wasternack C. Hause B. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. Ann. Bot. 2013; 111(6): 1021–1058.
37. Rahman K.S.M. Development of a simple and low cost microbioreactor for high-throughput bioprocessing. Biotechnol. Lett. 2009; 31 (2), 209-214.