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Minimizing adverse effects of drought stress on maize (Zea mays L.) using foliar application of jasmonic and salicylic acids

Fatemeh Nasr, Yahya Emam*, and Afshin Zamani

Department of Plant Production and Genetics, School of Agriculture, Shiraz University, Shiraz 7155713876, Iran.
*Corresponding author; yaemam@shirazu.ac.ir

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Abstract

Objective: Drought stress is a major factor limiting the growth and yield of maize (*Zea mays* L.). This experiment aimed to examine the effects of foliar application of salicylic acid (SA) and methyl jasmonate (MJ) in both single and combined concentrations on morphological, physiological, and biochemical traits of maize plants under varying levels of water deficit.

Methods: The experiment was carried out in the greenhouse of the School of Agriculture, Shiraz University, Iran, using a completely randomized design with three irrigation levels (100, 75, and 50% of full irrigation, FI) and nine foliar spray treatments (control, SA 0.5 and 1 mM, MJ 10 and 20 µM, and four SA and MJ combinations) in 2022. Growth traits such as shoot height, stem diameter, leaf area, as well as fresh and dry weights of shoots and roots, SPAD index, and chlorophyll content (chlorophyll a, b, and total), along with biochemical traits including anthocyanin, hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) were measured.

Results: Drought stress resulted in a notable decline in vegetative growth and an elevation in oxidative indices; however, the combined treatments effectively mitigated these adverse effects. In severe drought (FI 50%), root dry weight increased from 2.0 g in the control to 4.6 g in the SA1&MJ20 treatment. Leaf area, which was less than 400 cm^2 in the control, was found to be 487 and 456 cm^2 with SA0.5&MJ10 and SA0.5&MJ20, respectively. The SA1&MJ10 combination maintained total chlorophyll above 1.2 mg g^{-1} FW under 100% FI conditions and preserved chlorophyll a at a higher level than the control during 70% FI. The combinations also resulted in the greatest reduction in H_2O_2 and MDA; under 50% FI, H_2O_2 and MDA levels decreased from 0.47 and 88 µmol g^{-1} FW in the control to 0.28 and 28.7 µmol g-¹ FW in the SA0.5&MJ10 treatment. Furthermore, the anthocyanin content in the combined treatments, particularly SA1&MJ20, reached levels exceeding 6.0 mmol g^{-1} FW, indicating an enhancement of secondary defense pathways and protection against reactive oxygen species. Similarly, the individual application of SA or MJ also exerted positive effects on several growth and biochemical traits depending on the irrigation level, indicating that single-hormone treatments also could be beneficial under specific FI conditions.

Conclusion: Reducing irrigation from 100% FI to 75% FI and 50% FI impaired growth and pigments and enhanced oxidative damage. Effects of SA and MJ were trait- and FI-dependent. Overall, SA&MJ (especially SA1&MJ20 and SA0.5&MJ10) tended to better support biomass under 50% FI, whereas MJ alone often maintained SPAD/chlorophyll more effectively under 75% FI. Thus, SA and MJ should be applied using a target-trait, FI-specific strategy, with co-application as a conditional option.

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Introduction

In recent decades, drought stress has been exacerbated by the increasing adverse effects of climate change, prompting strong efforts to develop strategies to mitigate the harmful impacts of drought stress on various crops (Bouras *et al.* 2019). These initiatives are part of global efforts to meet the rising demands for food, feed, and fiber (Izadi *et al.* 2025). Therefore, the development and application of sustainable, ecologically friendly, economically viable, and reproducible compounds like osmolytes and phytohormones offer a potential and positive strategy to alleviate the adverse effects triggered by drought stress (Ozturk *et al.* 2021). Phytohormones are essential internal signaling molecules that play a significant role in regulating various physiological and molecular processes essential for the establishment, development, and adaptation to stress conditions in plants (Izadi *et al.* 2025). During abiotic stresses such as drought, careful regulation of phytohormone signaling pathways is crucial for maintaining cellular balance and ensuring plant survival (Jogawat *et al.* 2021).

Drought stress generally leads to significant reductions in both root and shoot biomass across various plant species. This is primarily attributed to changes in water uptake and photosynthetic efficiency (Zamani *et al.* 2024; Jahani Doghozlou *et al.* 2025). These responses are comprehensively controlled by plant hormones such as abscisic acid, auxins, cytokinin, gibberellins, ethylene, jasmonic acid, and salicylic acid, which orchestrate intricate signaling pathways aimed at mitigating damage caused by stress and fostering adaptive mechanisms (Ozturk *et al.* 2021). Empirical data consistently show that changes in phytohormone levels or signaling processes can boost drought tolerance by enhancing water-use efficiency, maintaining cell turgor, and activating antioxidant defenses (Jogawat *et al.* 2021).

Jasmonic acid (JA) is a naturally occurring plant growth regulator in higher plants, where it plays important roles in growth, development, stress responses, and adaptation (Creelman and Mullet

1995). When biotic and abiotic stresses occur, biologically significant signaling molecules, including JA and its volatile methyl jasmonate (MJ), are synthesized, which play a significant role in plant protection and adaptation (Tayyab *et al.* 2020). MJ plays an important role in promoting drought tolerance by reducing oxidative stress, maintaining cellular integrity, and increasing overall plant resilience under water-deficient conditions (Creelman and Mullet 1995; Yang *et al.* 2017; Ashry *et al.* 2018).

Salicylic acid (SA; o-hydroxybenzoic acid) is a naturally occurring phenolic compound in higher plants that functions as a hormone-like signaling molecule. The role of SA is central to the regulation of various physiological and developmental processes, including flowering, germination, photosynthesis, regrowth, and membrane permeability (Shemi *et al.* 2021). In addition to its role in developmental processes, SA serves as a key mediator in plant defense against both biotic and abiotic stresses. Due to the positive effects of chemical inducers like SA and MJ, this work aimed to determine both the individual and combined roles of SA and MJ in maize plants under drought stress conditions.

Maize (*Zea mays L.*), originally from the New World, is an essential staple crop that sustains a large portion of the world's population. It has various dietary and industrial applications *(Slafer et al*. 2021). Environmental stresses, particularly drought, lead to severe crop losses that put farmers' incomes at risk and threaten food security within households. Understanding the exact impact of these stresses on maize yield is also crucial for developing measures that enhance the resilience and sustainability of the global food system (Carrera *et al.* 2024).

Due to the positive effects of chemical inducers like SA and MJ, the purpose of this experiment was to assess how foliar applications of MJ (10 and 20 µM), SA (0.5 and 1 mM), and their combinations on maize plants influence morpho-physiological and biochemical traits under different water-deficit stress levels (100%, 75%, and 50% of full irrigation (FI)) at the vegetative growth stage.

Materials and Methods

Experimental setup

To explore the role of foliar application of SA and MJ on maize exposed to irrigation regimes, a pot experiment was planned in 2022 at the research greenhouse of the Plant Production and Genetics, School of Agriculture, Shiraz University, Shiraz, Iran (52° 58' longitude, 29° 72' latitude). The experiment consisted of a total of 81 pots (9 × 3 × 3) comprised of foliar applications of eight levels of SA, MJ, and their combinations, three irrigation levels with three replicates, arranged according to the completely randomized design.

The plant material used in this experiment was seeds of a single-cross hybrid maize (SC 704), which were obtained in 2021 and disinfected with the Raxil® fungicide, with 98% germination percentage. Seeds were planted in a loamy soil, the characteristics of which are presented in Table 1. Each pot was filled with 4.4 kg of soil. Fertilization was applied at two stages based on the results of the soil test. Before sowing, the soil received 20.15 g of triple super phosphate in 2 L of water and 47.82 g of urea in 2 L of water, each applied separately at 20 ml per pot. At the V3 stage (three leaves), 255 g of NPK 20-20-20 was dissolved in 45 L of water, and each pot received 500 ml of the solution. The diameter of each pot was 20 cm, with a height of 18 cm, and four holes in the bottom to allow drainage. The total weight of each pot, along with gravel and soil, reached 5 kg. Before sowing, the location of the pots was sprayed with 25 ml of Cypermethrin insecticide in 5 L of water to repel rodent pests.

Table 1. Physical and chemical properties of the soil sample.

Physical properties	Values	Chemical properties	Values
Soil depth (cm)	0-30	EC (dS/m)	0.64
Texture	Clay Loam	pH	7.2
Sand (%)	29.6	T.N.V (%)	44
Silt (%)	24	O.C (%)	1.2
Clay (%)	46.4	T.N (%)	0.12
FC (%)	25.4	P.ava (mg/kg)	5
PWP (%)	10.6	K.ava (mg/kg)	198
BD (g/cm^3)	1.2	-	-

FC: field capacity; PWP: permanent wilting point; BD: bulk density; EC: electrical conductivity; pH: potential of hydrogen; T.N.V: total neutralizing value (percentage of neutralizing materials); O.C: organic carbon; T.N: total nitrogen; P.ava: available phosphorus; K.ava: available potassium.

Treatment application

The treatments including irrigation at three levels of 100%, 75% and 50% FI, foliar application consisted of a control (application of distilled water), MJ at two concentrations, 10 and 20 µM (MJ10 and MJ20, respectively), SA at two concentrations, 0.5 and 1 mM (SA 0.5 and SA10, respectively), and a combination of MJ and SA at four levels: SA at 0.5 mM and JA at 10 µM (SA0.5&MJ10), SA at 0.5 mM and MJ at 20 µM (SA0.5&MJ20), SA at 1 mM and MJ at 10 µM (SA1&MJ10), and SA at 1 mM and MJ at 20 µM (SA1&MJ20).

In each pot, four uniform seeds were planted at the same depth on July 26, 2022. The pots were irrigated daily. When the plants were at the two-leaf stage (V2), all the pots were thinned and reduced from four plants to three. After 17 days at the three-leaf stage (V3), the plants were sprayed with the appropriate growth regulator. Water stress was also applied 26 days after seed sowing at the five-leaf stage (V5). For this purpose, the field capacity (FC) of the soil was determined at the soil science laboratory. After that, the amount of irrigation in the FI treatment (FI 100%) was measured based on the weight of the pots at the field capacity weight (FCW) in the greenhouse. For medium and severe water stress treatments (75% and 50% FI), the FCW of the pots was multiplied by 0.75 and 0.5, respectively. Then all the pots were accurately irrigated until their weight reached the determined FCW. The final data collection was recorded at the seven-leaf stage (V7).

Measured characteristics

To determine the morphological and physiological characteristics of the plants, various traits were measured. The plants' shoot height was measured as the distance between the plant collar and the tip of the tallest leaf in all three plants in each pot, and their average was recorded. Stem diameter was measured with calipers for all three plants in each pot, and the average was recorded in centimeters (cm). Other morphological characteristics were measured after the plants were harvested. The average shoot fresh weight of the aerial part was measured. Roots were then immersed in a container full of tap water, and the roots were gently washed of any visible soil particles. Clean roots were blotted dry with paper towels and immediately weighed for root fresh weight, then dried at 75 °C for 48 h to determine root dry weight. The leaf area was measured based on Pearce's method. Based on this method, the maximum length (cm) and width (cm) of each leaf were measured with a ruler, and the area was calculated by multiplying length and width by a fixed factor of 0.75 (Pearce *et al*. 1975). The SPAD for three leaves of the same age was recorded with a chlorophyll meter.

Sample collection and measurement of biochemical characteristics

Leaf samples were cut with sterilized scissors and placed in labeled aluminum foil. The samples were then frozen in liquid nitrogen and transferred to a freezer at -80 °C for further measurements.

Measurement of chlorophyll a, chlorophyll b, and total chlorophyll: Arnon's standard method was used to measure the amount of chlorophylls and carotenoids (Arnon, 1949). At first, the acetone extract was homogenized with 0.5 g of leaf sample in 5 ml of 80% acetone and 0.3 g of calcium carbonate (to prevent precipitation and pheophytin). Then, the absorbance of the soluble extract was read at three wavelengths of 646, 663, and 470 nanometers (nm), respectively, for chlorophyll a, b, and carotenoids with an ELISA reader.

Chlorophyll *a* (mg/ml) = (0.0127 × OD663) – (0.00269 × OD645)

Chlorophyll *b* (mg/ml) = (0.0229 × OD645) – (0.00468 × OD663)

Chlorophyll *a* + Chlorophyll *b* (mg/ml) = (0/0202 × OD645) + (0/00802 × OD 663)

OD: Optical density at the specified wavelength

V: Volume of the extracted sample (ml)

W: Fresh weight of the sample (g)

Anthocyanin content: To determine the anthocyanin content in the leaves, a methanolic extract was prepared. For the preparation of this extract, leaf samples were homogenized in acidic methanol (HCl: Methanol, 1:99, v/v) and kept in the dark for 24 hours. The ethanolic extracts were then centrifuged at 7000 rpm for 10 minutes, and the supernatants were collected. The absorbance of the samples was measured with a spectrophotometer at wavelengths between 529 and 650 nm (Havaux and Kloppstech 2001).

H_2O_2: To determine the H_2O_2 concentration, the method of Loreto and Velikova (2001) was employed with slight modifications. Absorbance at 390 nm was measured using a spectrophotometer, and the H_2O_2 concentration in the leaves was calculated based on the absorbance of the reaction mixture with different concentrations of standard.

MDA: The leaf sample was homogenized in trichloroacetic acid (TCA) solution. Then, the samples were centrifuged for 30 minutes at 13,000 rpm and 4 °C; Then, thio-barbituric acid (TBA) dissolved in 20% TCA was added to the separated supernatant after centrifugation; Then it was placed in a hot water bath with a temperature of 95 °C for 30 minutes and then, in an ice water bath, and the color of the samples turned orange. The amount of MDA in the samples was calculated by measuring the absorbance at 532 and 600 nm with a spectrophotometer and with the extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer 1968).

Statistical analysis

Collected data on morphophysiological traits were first assessed for normal distribution with the Shapiro-Wilk test (Shapiro and Wilk 1965) with SAS v. 9.4 software (SAS Institute Inc.). The data were then analyzed and evaluated by analysis of variance under the general linear model as described by Steel and Torrie (1960). The least significant difference (LSD) test was employed as a *post-hoc* test to identify significant differences, with a *p*-value of ≤ 0.05, considered statistically significant.

Results

Shoot height

The effect of water-deficit stress, plant growth regulators, and their interaction on shoot height was significant at $p \leq 0.01$ (Table 2). Reducing irrigation to 75% and 50% FI resulted in a significant decrease in shoot height. At 75% FI, shoot height declined by approximately 20% relative to the control with full irrigation, whereas at 50% irrigation, the reduction approached 30% (Figure 1).

Table 2. Analysis of variance for morpho-physiological traits of maize (*Zea mays* L.) in response to plant growth regulators under irrigation treatments.

SOV	df	SH	SD	LA	SFW	RFW	RDW	SPAD
WS	2	8944.62**	0.85**	3526909.28**	20887.18**	1348.53**	5.97**	538.64**
PGRs	8	56.57**	0.02**	77050.59**	167.03**	27.12**	3.05**	7.68**
WS×PGR	16	41.90**	0.01**	25768.74**	357.57**	104.44**	2.82**	4.57**
Error	54	3.63	0.0004	2544.06	7.73	2.52	0.13	0.37
CV (%)	-	2.03	2.45	6.72	6.65	10.51	13.88	1.44

WS: Water-deficit stress; PGRs: Plant growth regulators; CV: Coefficient of variation; SH: Shoot height; SD: Stem diameter; SFW: Shoot fresh weight; RDW: Root dry weight; RFW: Root fresh weight; SPAD: Chlorophyll index; LA: Leaf area; ns, *, **: Not significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

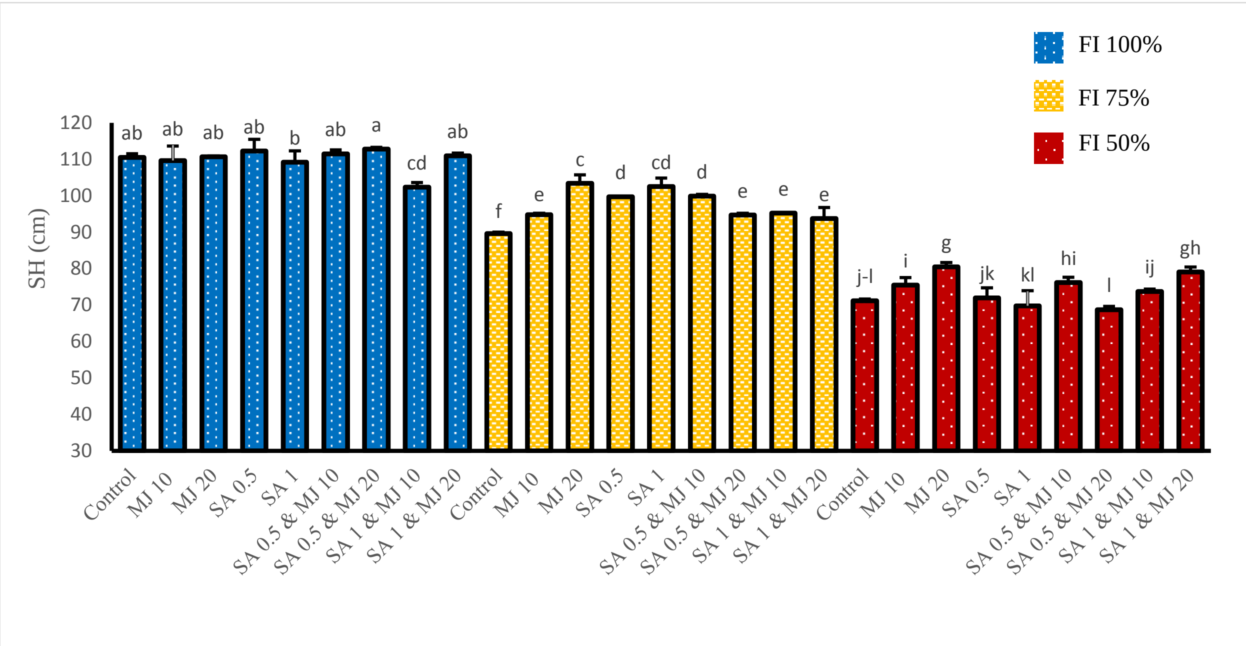

Figure 1. The interaction effect of irrigation treatments and plant growth regulators on maize shoot height (SH); Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

At 75% FI, applying JA at a concentration of 20 µM (MJ20) significantly promoted stem elongation, resulting in an average height of 103.66 cm. The finding emphasizes the significant role of MJ20 in fostering increased height under moderate drought stress conditions. This may be achieved through the activation of stress response pathways related to jasmonate, which facilitate cellular enlargement and improve water use efficiency. However, combination treatments (e.g., SA0.5&MJ10) also led to greater shoot height compared to the drought control that received no growth regulator treatment. Still, their effectiveness decreased compared to MJ20 alone. This suggests possible interference or breakdown of signaling pathway synergy under moderate water limitation.

Severe drought stress (FI 50%), resulted in a significant decrease in shoot height compared to well-watered conditions. MJ20, however, continued to perform at the highest level, maintaining a maximum shoot height (80.46 cm) among all treatments. The treatment SA0.5&MJ20 demonstrated less effectiveness, indicating a lower protective ability.

Stem diameter

Water deficiency, plant growth regulators, and their interaction significantly affected stem diameter ($p \leq 0.01$, Table 2). For 75% FI, stem diameter decreased by about 10% to 15% compared to the full-irrigated control (FI 100%), while a larger reduction, from 30% to 40%, was observed for 50% FI (Figure 2). This indicates a high sensitivity of vessel development to irrigation levels. Under 100% FI, SA0.5&MJ10 resulted in the greatest increase in stem diameter (1.21 cm), surpassing all other treatments.

For 75% FI, MJ10 alone was most effective in maintaining stem diameter, with its values nearly matching those of the non-stressed control (1.04cm). The other individual or combined treatments at this level of irrigation showed no significant differences, indicating that while MJ greatly reduces stress-induced stem thinning, combining it with other treatments under moderate stress does not produce additive or synergistic effects. In severe water deficiency (FI 50%), there was a significant decrease in stem diameter by all treatments in comparison to full irrigation. However, MJ10, MJ20, and SA0.5 were found as the most efficient treatments in maintaining stem thickness when conditions are very harsh (0.74, .73, and 0.74, respectively).

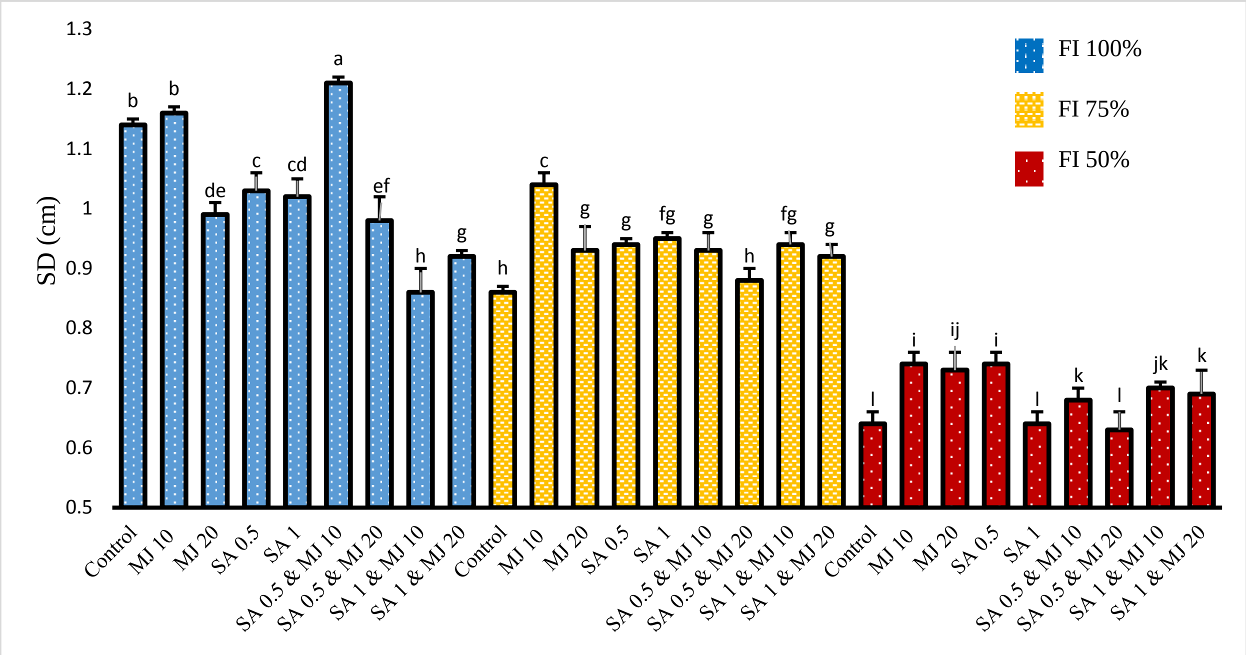

Figure 2. The interaction effect of irrigation treatments and plant growth regulators on maize plant stem diameter (SD); Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

Leaf area

While irrigation decreased from 100% to 75% and 50% FI, there was a significant reduction in leaf area, indicating that canopy establishment is sensitive to available water (Figure 3).

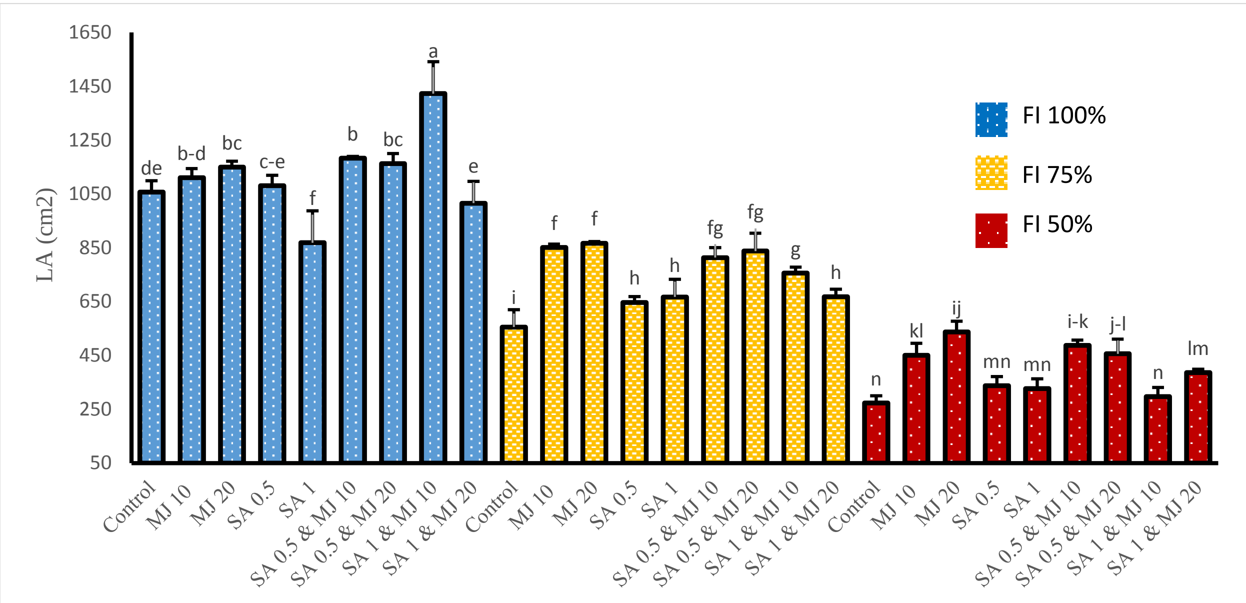

Figure 3. The interaction effect of irrigation treatments and plant growth regulators on maize leaf area (LA); Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

Under 100% FI, SA1&MJ10 produced the highest leaf area (1,423 cm²). In contrast, SA1 alone resulted in a smaller leaf area (868.84 cm²), which was even less than that of the untreated control, indicating limited activity or potential inhibitory effects of SA1 when applied by itself under non-stress conditions.

Under 75% FI conditions, plants treated with MJ alone and SA0.5&MJ10, SA0.5&MJ20, and SA1&MJ10 formed an intermediate statistical group with leaf area values between 800 and 950 cm², showing partial stress compensation. In contrast, SA1 alone and SA1&MJ20 resulted in lower leaf area values and reduced stress response under moderate water deprivation.

Under severe drought stress (FI 50%), leaf area decreased significantly across all treatments. Nonetheless, treatments such as MJ10, MJ20, SA0.5&MJ10, and SA0.5&MJ20 notably surpassed single SA and control treatments in preserving leaf area. Indeed, there was no significant increase in leaf area with SA alone at any level of irrigation. Overall, under both moderate and severe stress, single applications of MJ or its combined applications with 0.5 mM SA proved superior to other applications in reducing the decline in leaf area.

Shoot fresh weight

Shoot fresh weight was significantly affected by irrigation regimes, growth regulators, and their interaction ($p \leq 0.05$, Table 2). Under full irrigation, SA1&MJ20 and the control treatment achieved maximum shoot fresh weight (85 g) (Figure 4).

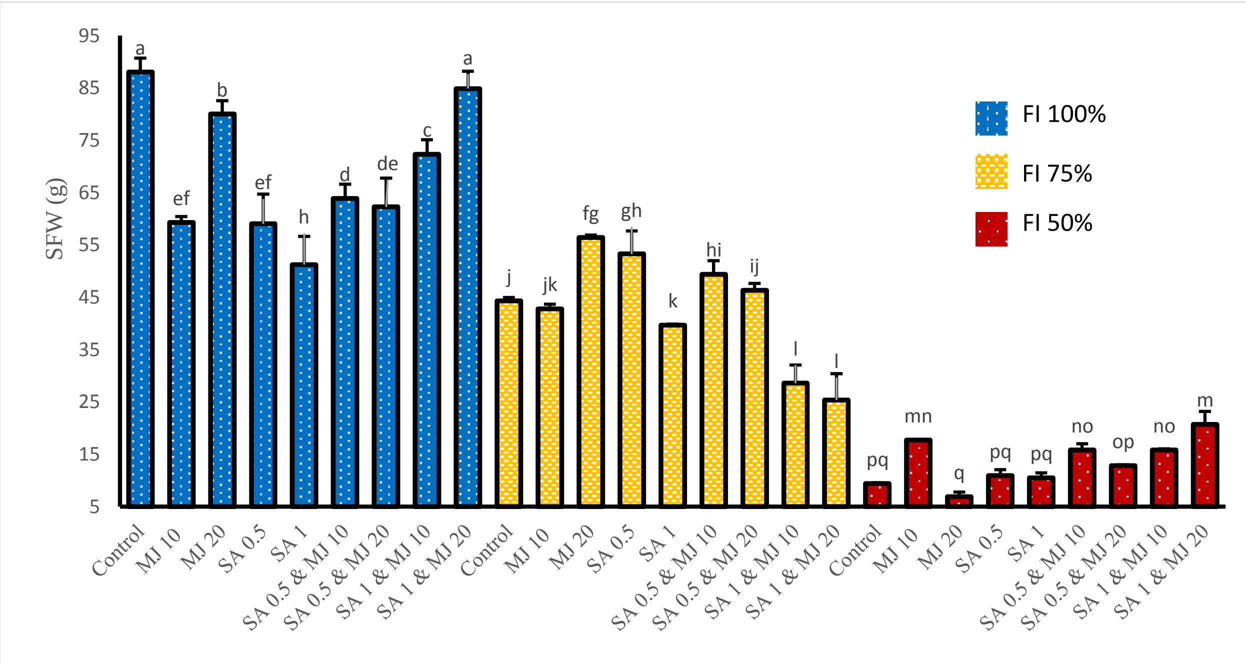

Figure 4. The effect of irrigation treatments and plant growth regulators on maize shoot fresh weight (SFW); Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

For 75% FI, the most effective was MJ20, maintaining shoot fresh weight above 56 g, thereby mitigating moderate drought stress. When compared, combined applications like SA1&MJ10 and SA1&MJ20 showed considerably lower values than 26 g, indicating possible antagonist interactions or dose-inhibited effects at this level of stress.

For severe water stress (FI 50%), significant reductions in shoot fresh weight occurred across all treatments, although SA1&MJ20 (20.76 g) and MJ10 (17.73 g) were most effective in maintaining shoot fresh weight, while only 9.48 g was recorded in the untreated control. These results demonstrate higher efficiency of MJ- and SA-based treatments in preventing shoot dehydration and biomass loss caused by drought.

Root fresh weight

Root fresh weight was significantly affected by irrigation regimes, growth regulators, and their interaction ($p \leq 0.01$, Table 2). Decreasing irrigation from 100% to 50% FI led to a notable reduction in root fresh weight (Figure 5).

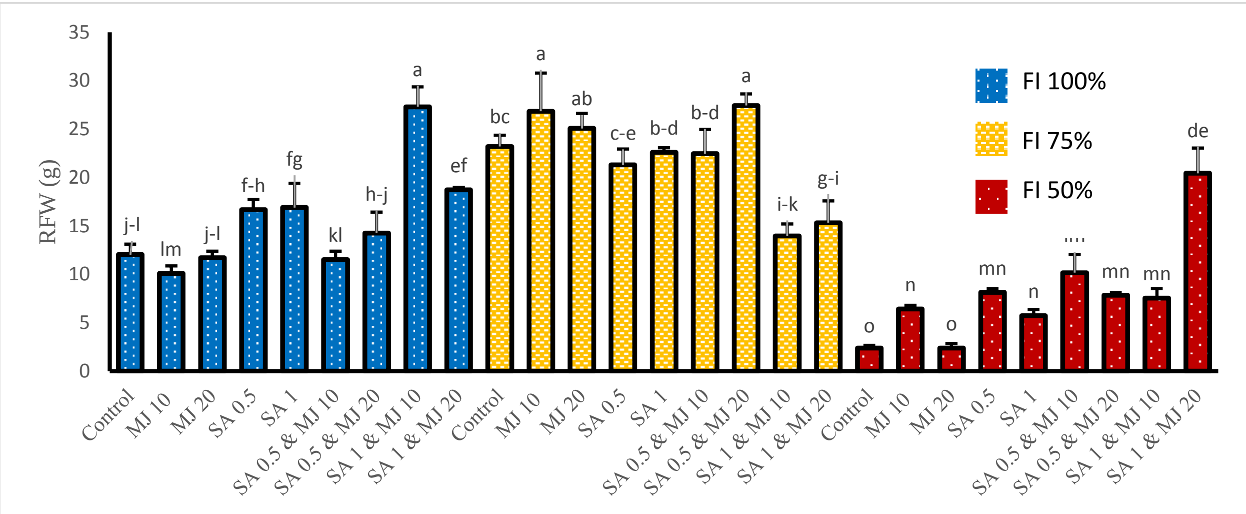

Figure 5. The interaction effect of irrigation treatments and plant growth regulators on maize root fresh weight (RFW); Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

Under optimal irrigation, SA1&MJ10 treatment achieved a maximum root fresh weight of over 27 g, significantly surpassing other treatments.

Under 75% FI, treatments such as MJ20, MJ10, and SA0.5& MJ20 had root fresh weight exceeding 25 g, values that are higher than some full irrigation treatments. This suggests a strong protective or priming effect of these treatments in maintaining root function under moderate stress conditions.

Under 50% FI, there were declines in root fresh weight across all treatments. Nonetheless, SA1&MJ20 remained superior, maintaining root fresh weight above 20 g. The sustained effectiveness under severe drought strongly indicates its significant role in drought resistance, likely by promoting greater root system vigor, osmotic adjustment, and hormonal signaling crosstalk. These findings highlight the potential of well-calibrated SA–MJ combinations in protecting root tissues against severe dehydration stress.

Root dry weight

Root dry weight was significantly affected by irrigation regimes, growth regulators, and their interaction ($p \leq 0.05$, Table 2). Overall, reduced irrigation decreased root dry weight, although several treatments successfully maintained or increased root biomass under water stress (Figure 6).

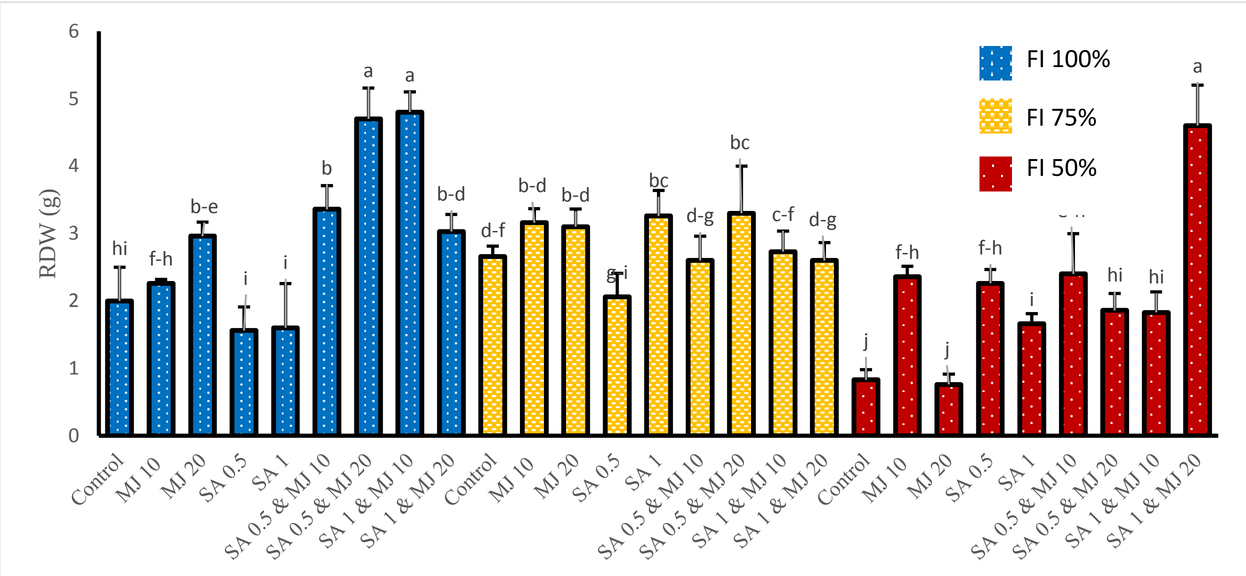

Figure 6. The interaction effect of irrigation treatments and plant growth regulators on maize root dry weight (RDW); Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

Under full irrigation (FI 100%), the SA1&MJ10 and SA0.5&MJ20 treatments had the highest root dry weight values (8.4 g and 7.4 g, respectively). In contrast, applications of salicylic acid alone resulted in the lowest root dry weight, at 1.6 g, which was lower than the untreated control at 2.0 g. These results confirm that, when used alone, salicylic acid is not very effective at increasing root biomass under non-stressed conditions; however, the combined use with methyl jasmonate has a synergistic effect.

Relatively high levels of root dry weight were maintained in 75% FI with SA0.5&MJ20 (3.30 g) and SA1 (3.26 g) treatments under moderate drought stress, while SA0.5 again ranked among the poorest treatments (2.06 g), as its protective ability was not very evident when used alone.

Under severe drought (FI 50%), SA1&MJ20 had the highest root dry weight (4.6 g), showing a strong, synergistic protective effect against water scarcity, likely due to improved root resilience and water uptake. The combination of SA1 and MJ20 consistently showed superior efficacy across all irrigation levels, demonstrating its potential to improve root biomass stability under both optimal and deficit conditions.

Chlorophyll index (SPAD)

Water-deficit stress negatively impacted the leaf chlorophyll index, based on SPAD values ($p \leq 0.05$, Table 3). There was about a 10% moderate decrease in SPAD values under 75% FI, while values declined by over 25% under 50% FI compared to the 100% FI (Figure 7).

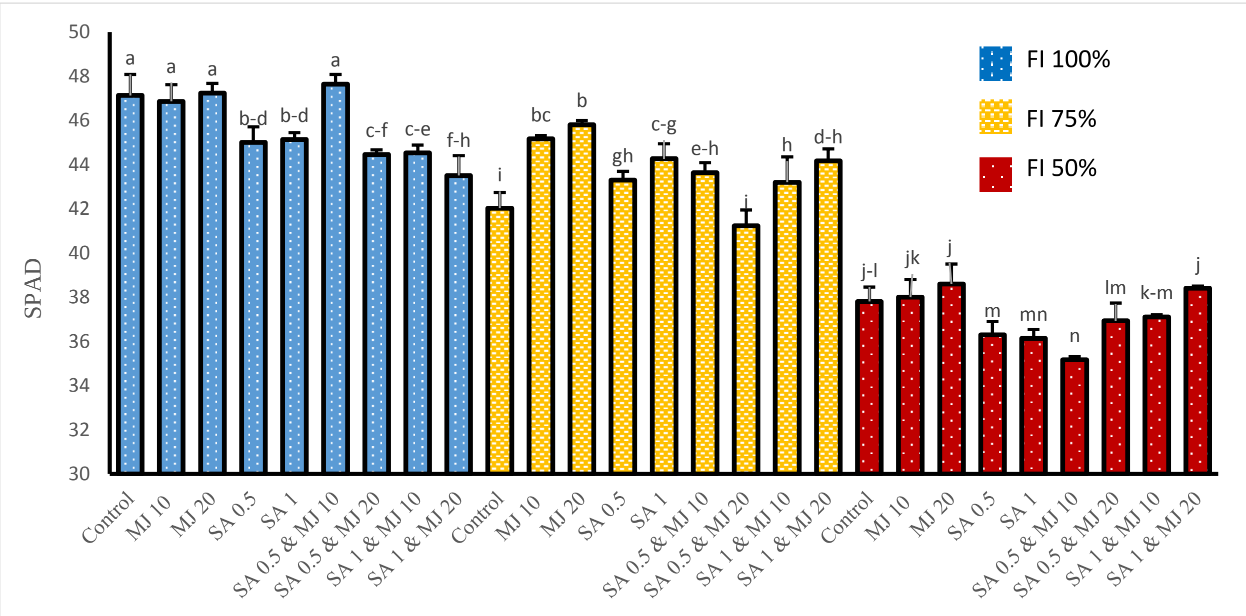

Figure 7. The interaction effect of irrigation treatments and plant growth regulators on maize SPAD value; Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

Table 3. Analysis of variance for biochemical traits of maize (*Zea mays* L.) in response to plant growth regulators under water-deficit stress regimes in greenhouse conditions.

SOV	df	Chl *a*	Chl *b*	T Chl	AC	H_2O_2	MDA
WS	2	0.01**	0.01**	0.02**	6.45**	0.04**	3525.17**
PGRs	8	0.04**	0.008**	0.09**	3.83**	0.04**	2592.82**
WS×PGR	16	0.04**	0.004**	0.08**	1.63**	0.04**	660.43**
Error	54	0.002	0.0008	0.005	0.27	0.001	66.27
CV (%)	-	6.33	9.52	6.99	12.21	14.23	14.52

WS: Water-deficit stress, PGRs: Plant growth regulators, Chl *a*: Chlorophyll *a*, Chl *b*: Chlorophyll *b*, T Chl: Total chlorophyll, AC: Anthocyanin content, H_2O_2: Hydrogen peroxide, MDA: Malondialdehyde; **: Significance at $p \leq 0.01$).

Under full irrigation, MJ10 and MJ20 had similar SPAD values, and SA0.5&MJ10 performed well, matching the control (47.00). When combined applications like SA1&MJ10 or SA1&MJ20 were used, reduced SPAD values indicated possible over-regulation or stress responses.

Under 75% FI, MJ10 and MJ20 kept outperforming other treatments with mean SPADs close to 45, showing they effectively preserve chlorophyll under moderate stress. Under 50% FI, the adverse effects of water-deficit stress on chlorophyll content become clear, as there was a sharp decline in SPAD values in treatments. Most of the regulatory treatments were not able to effectively promote chlorophyll conservation under these severe conditions. The SA0.5, SA10, and SA0.5&MJ10 treatments specifically showed lower or similar SPAD values compared to the untreated control experiencing drought stress. These treatments are likely to have inhibitory effects when used under severe water deficiency.

Chlorophyll a (Chl a)

Chl a concentration was significantly affected by irrigation regimes, plant growth regulators, and their interaction ($p \leq 0.05$, Table 3). As shown in Figure 8, the levels of Chl a markedly decreased when irrigation was reduced, especially under severe stress conditions (FI 50%), where untreated controls showed a significant decline (<0.5 mg g^{-1} FW). However, different growth regulators were shown to effectively mitigate this decline.

Under full irrigation (FI 100%), MJ10, SA0.5, and the control treatment contained the highest levels of Chl a, significantly exceeding 0.80 mg g^{-1} FW. The lowest pigment level was observed with MJ20 (0.53 mg g^{-1} FW), which was even lower than in the untreated control.

For moderate drought (FI 75%), MJ10 responded most favorably and achieved 1.02 mg g^{-1} FW in Chl a, higher than in the fully irrigated control. This notable increase may suggest an upregulation

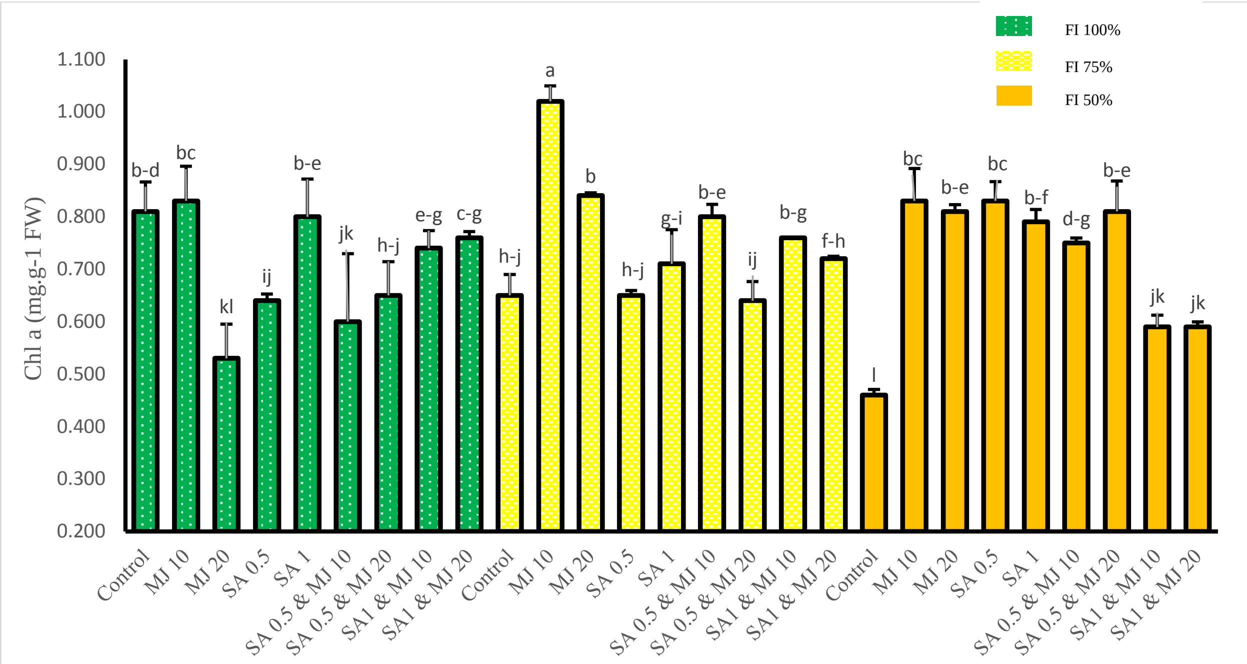

Figure 8. The interaction effect of irrigation treatments and plant growth regulators on maize plant Chlorophyll a (Chl a); Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

of chlorophyll biosynthesis or a slowdown of degradation processes induced by jasmonate signaling under moderate water stress.

Under 50% FI, there was an increase of 30% or more in Chl a compared to the control treated with severe drought stress. These results highlight the potential of specific regulator treatments in maintaining the stability of photosynthetic pigments under water limitation, presumably through regulation of oxidative stress reactions and the preservation of a functional chloroplast membrane system.

Chlorophyll b *(*Chl b*)*

A common trend showing a decrease in Chl b was observed as drought severity increased; however, different treatments effectively mitigated these declines across various irrigation systems (Figure 9).

Under 100% FI, MJ10 exhibited the highest concentration of Chl b (0.38 mg g^{-1} FW), followed by SA1 (0.37 mg g^{-1} FW), and SA0.5&MJ10 (0.37 mg g^{-1} FW). For 75% FI, there was an increase in Chl b during stress control through the application of growth regulators, although in most cases, combined treatments were not as effective as individual applications.

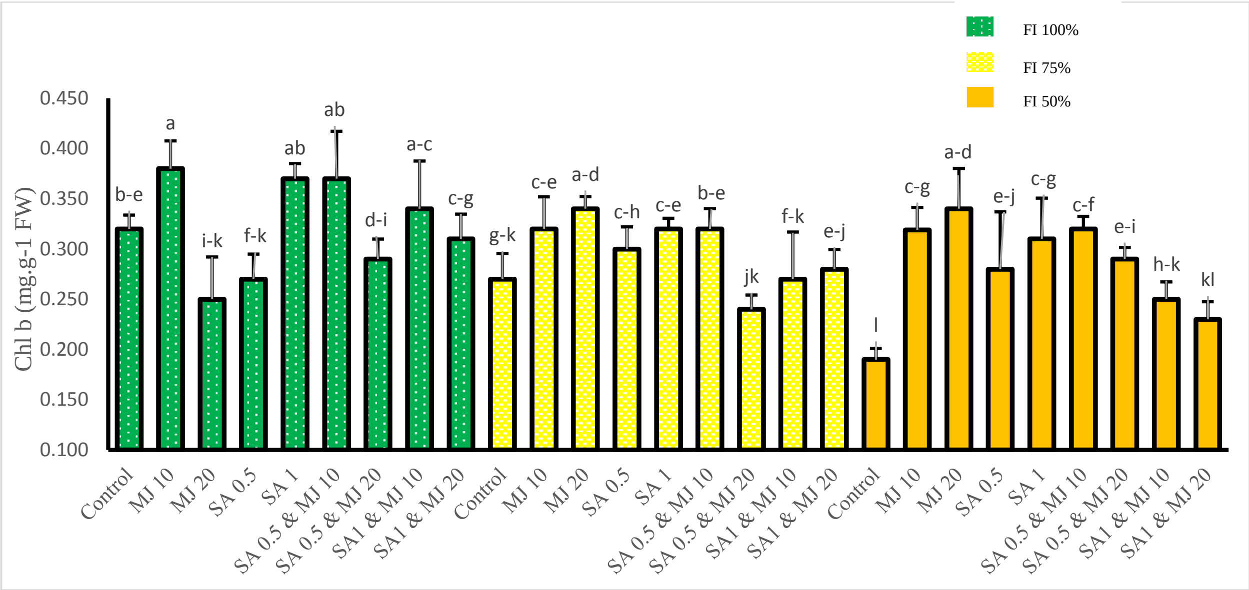

Figure 9. The interaction effect of irrigation treatments and plant growth regulators on maize plant Chlorophyll b (Chlorophyll b); Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

For 50% FI, the untreated control showed the lowest Chl b (0.19 mg g^{-1} FW), indicating a significant decline in photosynthetic pigment stability. Notably, the MJ20 treatment exhibited the highest Chl b at this stress level (0.34 mg g^{-1} FW). This result highlights the role of MJ in enhancing pigment preservation during dehydration, presumably through stress-responsive gene upregulation and activation of antioxidants that protect against loss of chloroplast structure.

Total chlorophyll

Total chlorophyll content was significantly affected by irrigation levels, plant regulators, and their interaction (Table 3). Under full irrigation (FI 100%), the highest total chlorophyll content was achieved with individual treatment of 10 µM MJ (1.20). This was statistically similar to the results with 1 mM SA1 and SA1&MJ10 treatments (Figure 10).

Under moderate drought (FI 75%) and severe drought (FI 50%), MJ10 and resulted in the highest total chlorophyll content (1.43), significantly higher than other treatments. This suggests a dominant role of MJ signaling during moderate water-deficit stress, potentially by enhancing antioxidant defense and delaying chlorophyll breakdown.

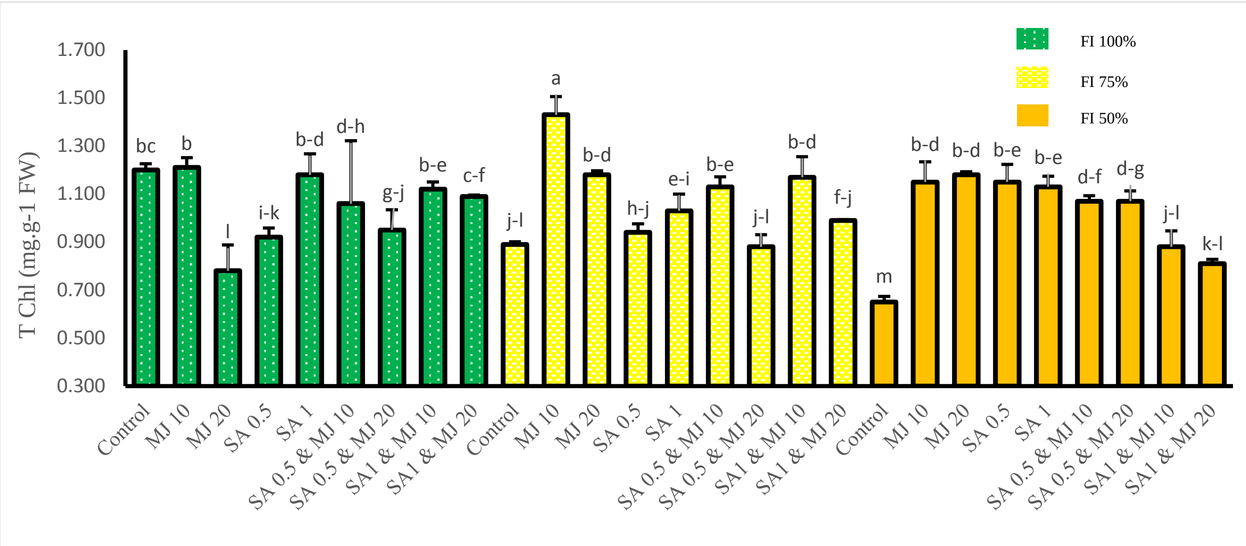

Figure 10. The interaction effect of irrigation treatments and plant growth regulators on maize plant total chlorophyll (T Chl); Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

Anthocyanin content

The anthocyanin content was significantly affected by different irrigation regimes, plant growth regulators, and their interaction ($p \leq 0.05$, Table 3). 50% FI generally caused a notable increase in anthocyanin levels, aligning with its role as a secondary metabolite that offers protective functions in antioxidative and photoprotective defense reactions of a plant under abiotic stress.

Under 100% FI, MJ20 exhibited the highest anthocyanin, at 5.06 mmol·g^{-1} FW, and was very close to being surpassed by SA1&MJ20, which recorded 4.89 mmol·g^{-1} FW. The lowest anthocyanin was observed in SA1&MJ20 and SA1 treatments (Figure 11). Lower anthocyanin levels may indicate decreased stress signaling or potential inhibitory hormonal interactions when plants are grown under optimal conditions, where defense mechanisms are naturally reduced.

Under 75% FI, SA1 showed the greatest value (5.19 mmol·g^{-1} FW), suggesting that SA can enhance secondary metabolism under mild stress. In contrast, most of the combined treatments showed smaller increases, indicating negligible additive effects under moderate stress levels. 50% FI further increased anthocyanin in most treatments. The SA0.5 and MJ20 treatments reached peak values of anthocyanin (>6.0 mmol·g^{-1} FW), highlighting their potential to reduce oxidative damage through reactive oxygen species (ROS) scavenging via anthocyanin. The other treatments also achieved higher anthocyanin levels compared to controls, reinforcing the role of external regulators in priming plant defense responses against severe water stress.

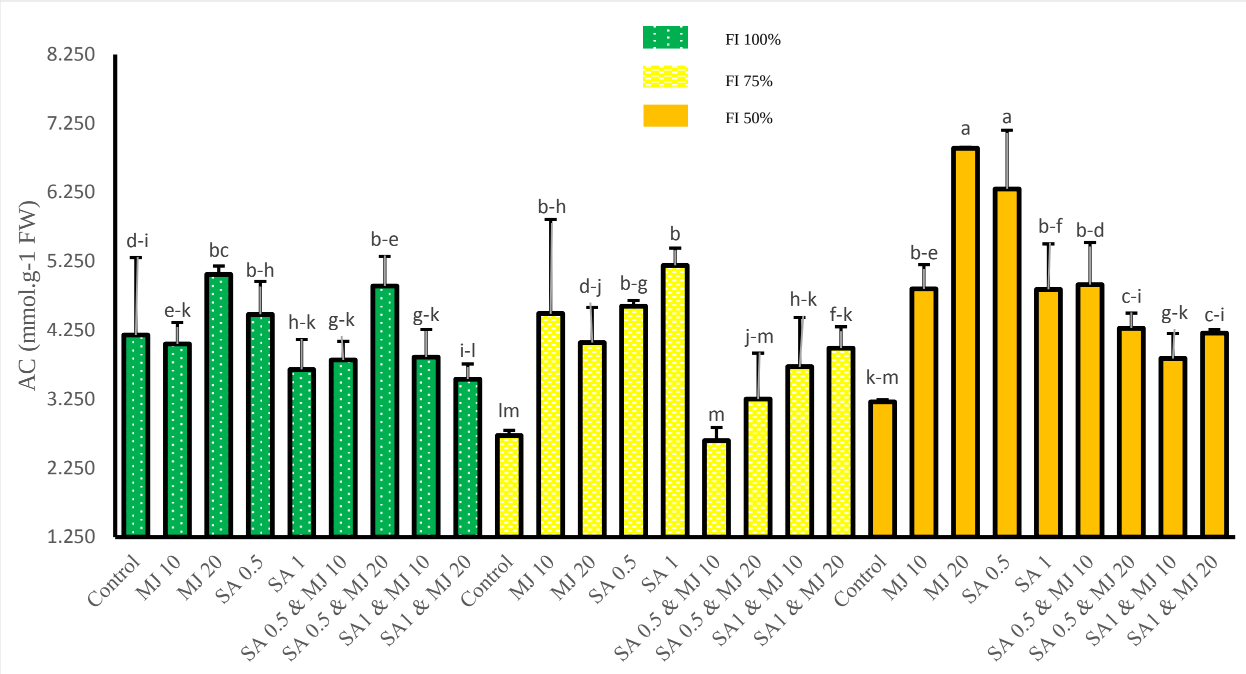

Figure 11. The interaction effect of irrigation treatments and plant growth regulators on maize plant anthocyanin (AC) content; Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

H_2O_2

H_2O_2 accumulation in leaf tissues was significantly influenced by irrigation levels, growth regulators, and their interaction ($p \leq 0.05$, Table 3). As a well-established marker of oxidative stress, H_2O_2 content increased with drought severity, indicating heightened ROS production under severe water-deficit conditions.

Under 100% FI, SA1&MJ10 and MJ20 induced the highest level of H_2O_2. As shown in Figure 12, it was significantly higher than the untreated control.

Under 75% FI, SA1&MJ10 produced the highest H_2O_2 content (0.50 μmol·g^{-1} FW), indicating strong activation of oxidative responses likely driven by a synergistic hormonal interaction under moderate stress. The only treatment that reduced H_2O_2 levels was MJ20, reaching a value of 0.05 μmol·g^{-1} FW; however, it was not significantly different from the control (0.09 μmol·g^{-1} FW).

Under 50% FI, the control treatment showed a high level of H_2O_2 (0.47 μmol·g^{-1} FW), while treating plants with growth regulators generally resulted in decreased H_2O_2 levels, indicating partial alleviation of oxidative damage. These findings suggest that ROS dynamics can be modulated by external regulators, such as MJ, to improve oxidative balance under extreme stress conditions.

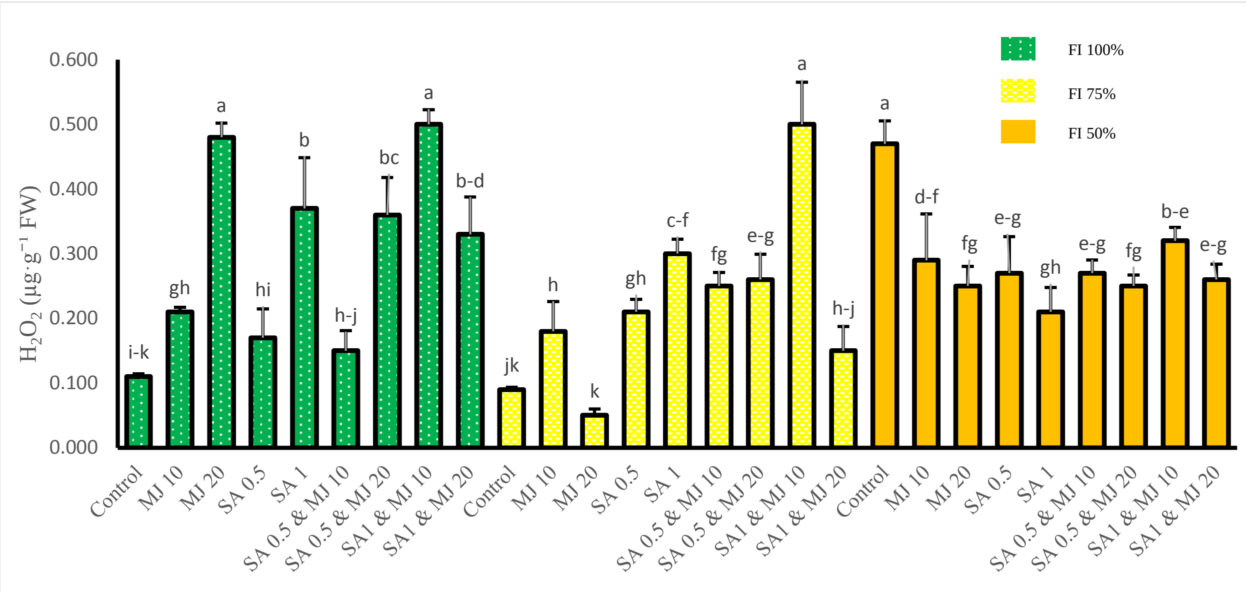

Figure 12. The interaction effect of irrigation treatments and plant growth regulators on maize hydrogen peroxide (H_2O_2) content; Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

MDA

The MDA content, a crucial marker of membrane lipid degradation and oxidative injury, was significantly influenced by irrigation regimes, plant growth regulators, and their interaction (Table 3). Regarding complete irrigation (FI 100%), the lower MDA levels were documented in SA0.5&MJ10 (23.98 µmol·g^{-1} FW) and SA1 (26.00 µmol·g^{-1} FW), demonstrating a significant reduction relative to untreated controls. These results substantiate the protective influence of SA, particularly when administered concomitantly with MJ, in mitigating oxidative damage and preserving cell membrane integrity under non-stressed conditions (Figure 13).

Under FI 75%, the minimal MDA content was recorded in the treatments SA0.5&MJ10 (31.60 µmol·g^{-1} FW) and SA0.5 (32.94 µmol·g^{-1} FW). This variation may result from complex interactions between stress perception mechanisms in plants and hormonal signaling pathways, which influence the dynamics of lipid peroxidation differently under moderate water limitation.

Under 50% FI, there was a sharp increase in MDA content in most treatments, with maximum values exceeding 88 µmol·g^{-1} FW found in the control treatment SA0.5&MJ20, SA1&MJ20, and MJ10. These results indicate significant membrane damage caused by increased oxidative stress. Conversely, SA0.5&MJ10 significantly reduced MDA content to 28.69 µmol·g^{-1} FW, demonstrating its effectiveness in reducing membrane damage and enhancing cell resistance under severe water stress.

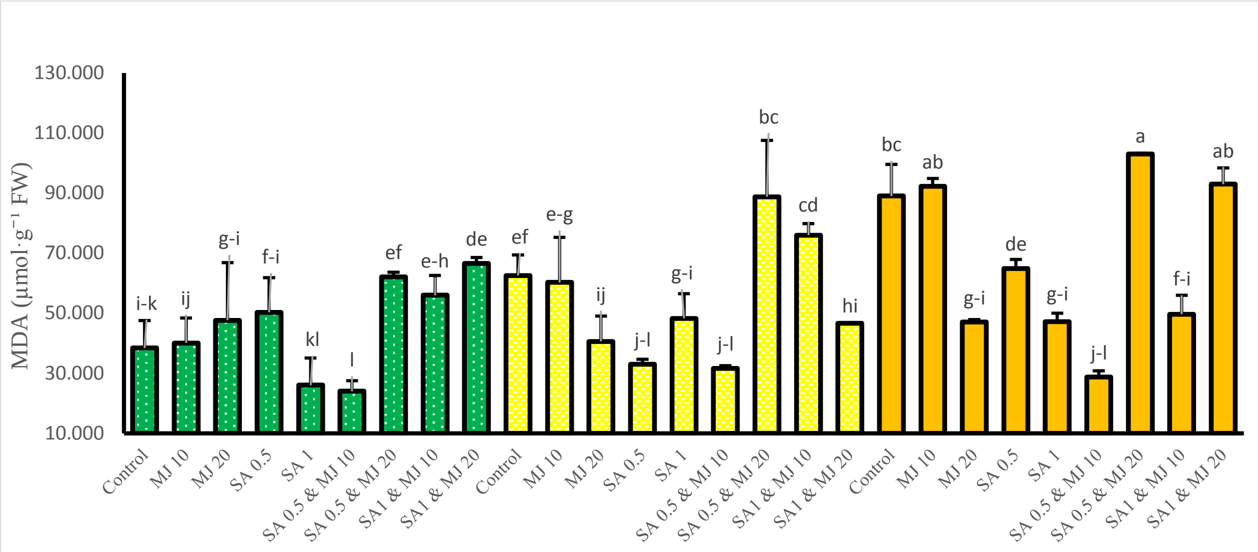

Figure 13. The interaction effect of irrigation treatments and plant growth regulators on maize malondialdehyde (MDA) content; Columns with different letters are significantly different at $p \leq 0.01$; MJ: Methyl jasmonate, SA: Salicylic acid, FI: Full irrigation.

Discussion

This experiment investigated how foliar applications of two plant growth regulators, namely SA and MJ in various concentrations—applied individually and together—affect the growth, morphological characteristics, and physiological responses of maize plants exposed to well-watered (FI 100%), moderate drought (FI 75%), and severe drought stress (FI 50%) conditions. The results clearly demonstrated that drought stress adversely affected the morphological, physiological, and biochemical traits of maize plants. Significant reductions in shoot height, stem diameter, leaf area, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight indicated impaired cell division, cell elongation, and nutrient uptake under conditions of reduced soil water content. The reduction in leaf area also caused a decrease in photosynthesis rate and slowed down carbohydrate supply to active organs, which ultimately limited overall plant growth and biomass accumulation. According to Jahani Doghozlou *et al.* (2025), photosynthesis, the primary process of carbon and energy production in plants, is greatly reduced by drought. It has been demonstrated that drought stress causes structural changes in chloroplasts (Ahmadi-Lahijani and Emam 2016), the buildup of protein-pigment complexes, and reduced transcription of genes involved in photosynthesis (Shazadi *et al.* 2024). These changes are accompanied by stomatal closure (Jogawat *et al.* 2021), reduced chlorophyll production (Bijanzadeh and Emam 2012), and inhibition of the Calvin cycle and electron transport chain (Amini *et al.* 2023). The present study also confirmed a significant decline in chlorophyll and the SPAD index. The roles of MDA and H_2O_2 in this decline are significant because the peroxidation of chloroplast membrane lipids led to pigment destruction and disrupted chlorophyll function.

Exogenous application of SA and MJ played a role in inhibiting chlorophyll degradation and decreasing MDA and H_2O_2 accumulation. MJ also contributed to pigment retention and organ weight gain by promoting cell elongation, root development, and stimulating jasmonate regulatory pathways. Sun *et al.* (2011) reported that MJ effectively enhances lateral roots in wild-type Arabidopsis by interacting with auxin signaling and biosynthesis pathways. In the combined treatments of SA and MJ, the increase in chlorophyll content along with the decrease in MDA indicated that these regulators can maintain membrane integrity and chloroplast stability under drought. Drought increased the production of ROS, including H_2O_2. The buildup of H_2O_2 caused lipid peroxidation and raised MDA levels, which are indicators of cell membrane damage. The present results demonstrated that the combined treatment of SA and MJ effectively reduces the levels of H_2O_2 and MDA, thereby protecting cells against oxidative damage. This effect was reportedly associated with an elevation in the activity of antioxidant enzymes (such as SOD, CAT, and POD) (Shah Jahan *et al.* 2019; Mustafa *et al.* 2021; Serna-Escolano *et al.* 2021) and the accumulation of osmolytes such as proline and soluble sugars (Sofy *et al.* 2020). Tayyab *et al.* (2020) reported that exposing maize plants to drought stress and applying SA and MJ with foliar treatment elicits numerous biochemical, morphological, and physiological responses, ultimately enhancing their tolerance to drought conditions.

The increase in anthocyanin content under severe drought conditions was also an important finding. In addition to its role as a secondary pigment, anthocyanin serves as an antioxidant and photoprotective agent and protects tissues against ROS (Chen *et al.* 2024). This study shows that using the SA and MJ combination significantly increased anthocyanin, indicating activation of secondary metabolic pathways and better osmoprotection under stress.

Another key issue was the dose dependence of responses to growth regulators. For example, evidence has indicated that high concentrations (2-3 mM) of SA suppressed drought tolerance and plant growth in wheat plants, while a low concentration (0.5 mM) enhances growth (Kang *et al.* 2012). Elevated levels of SA can be inhibitory or even toxic, as they cause excessive ROS production in photosynthetic tissues and increase oxidative damage (Miura and Tada 2014). On the other hand, Sun *et al.* (2011) reported that the effects of MJ on lateral root formation are concentration-dependent. While low concentrations promote lateral root development, higher concentrations may have inhibitory effects, likely due to disruptions in auxin transport or other hormonal crosstalk.

Conclusion

Reducing irrigation from 100% FI to 75% FI and 50% FI adversely affected maize morpho-physiological performance and increased oxidative damage indicators, confirming the sensitivity of maize at the vegetative stage to water limitation. Overall, the response to SA and MJ (single vs. combined) was trait- and stress-severity dependent, and no treatment was uniformly superior across all irrigation regimes. In general, combined SA&MJ treatments, particularly SA1&MJ20 and SA0.5&MJ10, provided stronger protection for several growth- and biomass-related traits, especially under 50% FI (e.g., improved root biomass and leaf area). However, under 75% FI, MJ alone (MJ10 and/or MJ20) was often more effective in maintaining SPAD and chlorophyll traits than combined applications. Moreover, combined treatments showed non-uniform effects on oxidative indices; notably, SA0.5&MJ10 reduced MDA under 50% FI, whereas some combinations (including SA1&MJ20) were associated with high MDA under severe stress. Therefore, SA and MJ should be applied using a target-trait and FI-specific approach, with SA&MJ co-application considered a promising but conditional strategy.

Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

References

Ahmadi-Lahijani MJ, Emam Y. 2016. Post-anthesis drought stress effects on photosynthesis rate and chlorophyll content of wheat genotypes. J Plant Physiol Breed. 6(1): 35-52. https://breeding.tabrizu.ac.ir/article_6269.html

Amini A, Majidi MM, Mokhtari N, Ghanavati M. 2023. Drought stress memory in a germplasm of synthetic and common wheat: antioxidant system, physiological and morphological consequences. Sci Rep. 13(1): 8569. https://doi.org/10.1038/s41598-023-35642-2

Ashry NA, Ghonaim MM, Mohamed HI, Mogazy AM. 2018. Physiological and molecular genetic studies on two elicitors for improving the tolerance of six Egyptian soybean cultivars to cotton leaf worm. Plant Physiol Biochem. 130: 224-234. https://doi.org/https://doi.org/10.1016/j.plaphy.2018.07.010

Bijanzadeh E, Emam Y. 2012. Evaluation of crop water stress index, canopy temperature and grain yield of five Iranian wheat cultivars under late season drought stress. J Plant Physiol Breed. 2(1): 23-33. https://breeding.tabrizu.ac.ir/article_3087.html

Bouras E, Jarlan L, Khabba S, Er-Raki S, Dezetter A, Sghir F, Tramblay Y. 2019. Assessing the impact of global climate changes on irrigated wheat yields and water requirements in a semi-arid environment of Morocco. Sci Rep. 9(1): 19142. https://doi.org/10.1038/s41598-019-55251-2

Carrera CS, Savin R, Slafer GA. 2024. Critical period for yield determination across grain crops. Trends Plant Sci. 29(3): 329-342. https://doi.org/10.1016/j.tplants.2023.08.012

Chen T, Xie L, Wang G, Jiao J, Zhao J, Yu Q, Chen Y, Shen M, Wen H, Ou X, *et al.* 2024. Anthocyanins-natural pigment of colored rice bran: composition and biological activities. Food Res Int. 175: 113722. https://doi.org/https://doi.org/10.1016/j.foodres.2023.113722

Creelman RA, Mullet JE. 1995. Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. Proc Natl Acad Sci USA. 92(10): 4114-4119. https://doi.org/10.1073/pnas.92.10.4114

Jahani Doghozlou M, Emam Y, Zamani A. 2025. The impacts of earlier flowering on grain yield of winter wheat cultivars under semi-arid conditions. Iran Agric Res. 44(1): 89-102. https://doi.org/10.22099/iar.2025.52009.1661

Jogawat A, Yadav B, Chhaya, Lakra N, Singh AK, Narayan OP. 2021. Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: a review. Physiol Plant. 172(2): 1106-1132. https://doi.org/https://doi.org/10.1111/ppl.13328

Kang G, Li G, Xu W, Peng X, Han Q, Zhu Y, Guo T. 2012. Proteomics reveals the effects of salicylic acid on growth and tolerance to subsequent drought stress in wheat. J Proteome Res. 11(12): 6066-6079. https://doi.org/10.1021/pr300728y

Liu Z, Ding Y, Wang F, Ye Y, Zhu C. 2016. Role of salicylic acid in resistance to cadmium stress in plants. Plant Cell Rep. 35(4): 719-731. https://doi.org/10.1007/s00299-015-1925-3

Miura K, Tada Y. 2014. Regulation of water, salinity, and cold stress responses by salicylic acid. Front Plant Sci. 5: 4. https://doi.org/10.3389/fpls.2014.00004

Mustafa T, Sattar A, Sher A, Ul-Allah S, Ijaz M, Irfan M, Butt M, Cheema M. 2021. Exogenous application of silicon improves the performance of wheat under terminal heat stress by triggering physio-biochemical mechanisms. Sci Rep. 11: 23170. https://doi.org/10.1038/s41598-021-02594-4

Ozturk M, Turkyilmaz Unal B, García-Caparrós P, Khursheed A, Gul A, Hasanuzzaman M. 2021. Osmoregulation and its actions during the drought stress in plants. Physiol Plant. 172(2): 1321-1335. https://doi.org/https://doi.org/10.1111/ppl.13297

Serna-Escolano V, Martínez-Romero D, Giménez MJ, Serrano M, García-Martínez S, Valero D, Valverde JM, Zapata PJ. 2021. Enhancing antioxidant systems by preharvest treatments with methyl jasmonate and salicylic acid leads to maintain lemon quality during cold storage. Food Chem. 338: 128044. https://doi.org/https://doi.org/10.1016/j.foodchem.2020.128044

Shah Jahan M, Wang Y, Shu S, Zhong M, Chen Z, Wu J, Sun J, Guo S. 2019. Exogenous salicylic acid increases the heat tolerance in Tomato (*Solanum lycopersicum* L) by enhancing photosynthesis efficiency and improving antioxidant defense system through scavenging of reactive oxygen species. Sci Hortic. 247: 421-429. https://doi.org/https://doi.org/10.1016/j.scienta.2018.12.047

Shazadi K, Christopher JT, Chenu K. 2024. Does late water deficit induce root growth or senescence in wheat? Front Plant Sci. 15: 1351436. https://doi.org/10.3389/fpls.2024.1351436

Shemi R, Wang R, Gheith EMS, Hussain HA, Hussain S, Irfan M, Cholidah L, Zhang K, Zhang S, Wang L. 2021. Effects of salicylic acid, zinc and glycine betaine on morpho-physiological growth and yield of maize under drought stress. Sci Rep. 11(1): 3195. https://doi.org/10.1038/s41598-021-82264-7

Slafer GA, Savin R, Pinochet D, Calderini DF. 2021. Wheat. In: Sadras VO, Calderini DF. (eds.) Crop physiology case histories for major crops. London: Academic Press, pp. 98-163. https://doi.org/10.1016/B978-0-12-819194-1.00003-7

Sofy MR, Seleiman MF, Alhammad BA, Alharbi BM, Mohamed HI. 2020. Minimizing adverse effects of Pb on maize plants by combined treatment with jasmonic, salicylic acids and proline. Agronomy. 10(5): 699. https://doi.org/10.3390/agronomy10050699

Sun J, Chen Q, Qi L, Jiang H, Li S, Xu Y, Liu F, Zhou W, Pan J, Li X, *et al.* 2011. Jasmonate modulates endocytosis and plasma membrane accumulation of the Arabidopsis PIN2 protein. New Phytol. 191(2): 360-375. https://doi.org/https://doi.org/10.1111/j.1469-8137.2011.03713.x

Tayyab N, Naz R, Yasmin H, Nosheen A, Keyani R, Sajjad M, Hassan MN, Roberts TH. 2020. Combined seed and foliar pre-treatments with exogenous methyl jasmonate and salicylic acid mitigate drought-induced stress in maize. PLoS One. 15(5): e0232269. https://doi.org/10.1371/journal.pone.0232269

Yang Z-B, He C, Ma Y, Herde M, Ding Z. 2017. Jasmonic acid enhances Al-induced root growth inhibition. Plant Physiol. 173(2) : 1420-1433. https://doi.org/10.1104/pp.16.01756

Zamani A, Emam Y, Edalat M. 2024. Response of bread wheat cultivars to terminal water stress and cytokinin application from a grain phenotyping perspective. Agronomy. 14(1): 182. https://doi.org/10.3390/agronomy14010182