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Research paper

Improving physiological performance and grain yield of maize by salicylic acid treatment under drought stress

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Abstract

A field experiment was conducted as a split-plot based on a randomized complete block design with three replications in 2020 to investigate the effect of different salicylic acid (SA) levels on physiological traits and grain, oil, and protein yields of maize (MV 524). The irrigation treatments were normal irrigation (irrigation after 60 mm evaporation), and irrigation disruptions from tassel emergence up to seed formation and from tassel emergence up to harvest maturity. The plants were sprayed with three levels of SA (1, 2, and 3 mM) and water at the tassel emergence stage. The irrigation and SA treatments were assigned to the main and subplots, respectively. Irrigation disruption at reproductive stages caused a decline in mean leaf water content (LWC), membrane stability index (MSI), chlorophyll content, leaf area index (LAI), grain yield, oil percentage, oil and protein yields, and an increment in leaf temperature and protein percentage, compared to normal irrigation. Application of SA, especially with 3 mM concentration, increased mean LWC, MSI, chlorophyll content, LAI, and grain, oil, and protein yields of maize. The highest positive correlation with grain, oil, and protein yields was recorded for LAI, followed by LWC and chlorophyll content index. These results suggest that water supply at reproductive stages is essential for successful maize production. Nevertheless, a foliar spray of 3 mM SA can improve the field performance and productivity of maize under normal and limited water availability.

Keywords: chlorophyll; drought stress; grain oil; growth regulator; maize

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Introduction

Maize (*Zea mays* L.) has a high capacity for converting light energy into chemical energy. This crop is widely cultivated in many countries due to its high productivity under different climatic conditions (Sah *et al*. 2020). Maize is widely used in the food industry to produce various products for human consumption and animal feed. Maize was recorded as the most-produced crop among cereals in the world, followed by wheat and rice (FAO 2021).

The occurrence of successive droughts due to limited water resources clarifies the necessity of investigating the responses of various crops to water shortages. Most of the arable lands in Iran are located in arid and semi-arid regions, where water is a scarce resource. Under such conditions, drought stress leads to significant reductions in crop yield, depending on rainfall quantity and distribution (Movahhedy-Dehnavy *et al*. 2009). This stress can limit the growth and yield of cereals such as maize. The availability of sufficient water at the vegetative and reproductive stages of maize is essential to produce an acceptable yield per unit area. The negative impacts of drought stress on the growth, development, and yield of maize depend on the severity of water deficit, developmental stage, and genotype (Ghassemi-Golezani *et al*.

2018b).

The limitation of water negatively affects dry matter accumulation by reducing leaf area and photosynthetic capacity of the plants (Ghassemi-Golezani *et al*. 2009). Other symptoms of drought stress include decreased leaf water content, loss of turgor pressure, and stomatal closure, leading to reduced cell and plant growth (Estaji and Niknam 2020). Increasing plant resistance to drought stress is possible through various methods such as plant breeding and the application of growth regulators. In comparison to the long-term and expensive plant breeding methods, the application of natural regulators such as salicylic acid (SA) is a comparatively simple and rapid technique for enhancing the stress tolerance of plants (Abbaspour and Rezaei 2014).

SA or ortho-hydroxybenzoic acid is a phenolic compound phytohormone that is involved in defense mechanisms to counter the negative effects of biotic and abiotic stresses (Abdoli and Ghassemi-Golezani 2021). This hormone has important effects on mineral uptake, membrane stability, stomatal performance, inhibition of ethylene synthesis, and improvement of plant growth (Ghassemi-Golezani *et al.* 2015). SA regulates many physiological processes involved in plant adaptation to environmental stresses. Foliar spray of SA increases leaf area and light interception thereby increasing the rate of net photosynthesis and grain yield (Ghassemi-Golezani *et al.* 2018a). Due to limited water resources and the widespread use of maize in the food industry, and animal feeding, this research aimed to find out the impacts of exogenous SA levels on some physiological traits related to drought tolerance and productivity of maize.

Materials and Methods

Experimental site

This research was performed in 2020 at the Research Farm of the University of Tabriz. This region is located at a longitude of 46˚ 17′E and a latitude of 38˚ 3′N with an altitude of 1360 m above sea level. This region has cold winters and hot summers. The average minimum and maximum air temperatures are 7.3 and 18.7 °C, respectively, and the average annual rainfall is about 280.75 mm. The soil physicochemical properties from a depth of 0- 30 cm of the soil are listed in Table 1.

Experimental design

A field experiment was conducted as a split plot design based on a randomized complete block design in three replicates. The irrigation treatments were normal irrigation (irrigation after 60 mm evaporation from class A pan), and irrigation disruptions (from tassel emergence up to seed formation and from tassel emergence up to maturity). The irrigation levels were allocated to main plots and SA treatments (1, 2 and 3 mM) and water spray (control)) were assigned to subplots.

Maize seeds (MV 524 hybrid, single ear, mid maturity) were obtained from Maxima Company. Each experimental plot had four rows with a length of 5 m, which were spaced 50 cm apart. The seeds were treated with 2 g kg^{-1} Benomyl, and then were sown in 19th May 2020 at a depth of 4-5 cm with a distance of 20 cm on a row to achieve a density of 10 plants m^{-2}. Then, 120 g urea (46% N) was added to each plot and then all plots were irrigated. Subsequent irrigations were performed after 60

Table 1. Soil physicochemical characteristics in the experimental area

Mineral components of soil (%)			Available elements (mg kg^{-1})					Total N (%)	Organic carbon (%)	EC (dS/m)	pH
Sand	silt	clay	Zn	Fe	Cu	K	P				
73	14	13	0.96	2.7	0.71	259	13.7	0.14	1.4	1.61	7.8

mm evaporation from the class A pan. More urea (120 g per plot) was added to the plots at the 7-8 leaves stage ($V_{(n)}$) of plants. Foliar spray of water and SA were carried out at vegetative (8-9 leaves) and reproductive (tassel emergence) stages.

Leaf water content (LWC): A leaf from the middle of a random plant in each plot was separated and its fresh weight was immediately determined. Then, this sample was dried in an oven at 75 °C for 48 h and weighed. LWC was calculated according to Ghassemi-Golezani et al. (2016a):

LWC = [(fresh weight - dry weight) / fresh weight] × 100

Leaf temperature (LT): LT was determined by an infrared thermometer (TES-1327). The LT of three upper, middle, and lower leaves of a plant from each plot were measured at the early grain-filling stage and their average was recorded.

Membrane stability index (MSI): The MSI was determined based on the electrical conductivity of leaf leachates in double-distilled water. Two fresh leaf samples of 0.2 g from three random leaves of each plot were weighed and placed in a test tube containing 20 ml of double-distilled water. Then a series of samples were placed in an oven at 40° C for 30 min (EC_1) and another series was exposed to 100 °C for 10 min (EC_2). Then, the electrical conductivity was measured with an electrical conductivity meter. The MSI was calculated according to Ghassemi-Golezani *et al.* (2016a):

MSI = $[EC_1 / EC_2] \times 100$

Chlorophyll content index (CCI): A portable chlorophyll meter CCM-200 (Opti-Sciences, Tingsboro, MA) was used to measure the leaf chlorophyll content index. Measurements were performed at the early grain-filling period. The chlorophyll content of mature and healthy upper, middle, and lower leaves of a plant from each plot was measured and the mean value was considered as the leaf chlorophyll index of each experimental unit.

Leaf area index (LAI): The leaves of a random plant from the middle part of each plot were separated at the early grain filling stage and the leaf area was measured by a leaf area meter (LI-COR, Model Li-3100C Area Meter, USA). The LAI was also calculated as:

LAI = Leaf area/Land area

Grain yield and oil and protein content: At seed maturity, the plants in 1 m^2 of each experimental plot (10 plants) were harvested. The seeds were then separated from the ear and the grain yield per unit area was recorded. The oil and protein percentages of the grains were determined separately using a seed analyzer (Zeltex ZX-50). The oil and protein yields were calculated as:

$$\text{Oil or protein yield (kg ha}^{-1}\text{) = Oil or protein percentage} \times \text{grain yield (kg ha}^{-1}\text{)}$$

Statistical analysis

The data were initially tested for normality and uniformity of variances and then were analyzed by MSTAT-C software. The mean data were compared by Duncan's multiple range test at $p \leq 0.5$. The figures were drawn by Excel software.

Results

Physiological traits

The effect of irrigation disruptions at reproductive stages on LWC, LT, MSI, chlorophyll content index, and LAI was significant. Foliar application of SA had also a significant impact on MSI, chlorophyll content index, and LAI (Table 2). The interaction of drought stress × foliar application was only significant for LAI (Figure 1).

Irrigation disruptions at reproductive stages significantly reduced LWC, MSI, and chlorophyll content index, but enhanced leaf LT. There was no significant difference in the LWC of plants under I_1 and I_2 and in LT and MSI under I_2 and I_3. Foliar application of SA, especially with 3 mM concentration, improved MSI and chlorophyll content index, compared to untreated plants (water spray). The difference between 2 mM and 3 mM concentrations was not statistically significant (Table 2). All SA concentrations similarly enhanced maize LAI under I_1 and I_2, but this positive effect was increased with increasing the SA concentration under prolonged drought stress during reproductive stages (Figure 1).

Grain yield

Grain yield per unit area was significantly affected by irrigation disruptions at reproductive stages and exogenous SA, but the interaction of these factors was not significant (Table 3). Drought stress during all reproductive stages reduced the grain yield of maize by about 37%. The SA spray, especially with 3 mM concentration, increased the grain yield of maize, compared to untreated plants (Table 3).

Oil and protein percentages and yields

The effect of irrigation disruptions at reproductive stages on grain oil and protein percentages and yields was significant. Exogenous SA had also a significant effect on these parameters (Table 3). The oil and protein percentage and oil yield were decreased by water stress at the reproductive stages. The means of these traits under I_1 and I_2 treatments were statistically similar. Drought stress during these stages significantly enhanced grain protein percentage. Foliar application of SA, especially with the 3 mM concentration, improved oil and protein yields, compared to untreated plants (water spray). The mean oil yield for 2 mM and 3 mM SA-treated plants was not statistically different (Table 3).

Correlation of coefficients

LWC, chlorophyll content index, and LAI were positively and significantly correlated with each other and also with grain, oil, and protein yields. However, the correlation of leaf temperature and protein percentage with all of the studied traits was

Table 2. Means ± SE of physiological traits for maize plants affected by irrigation disruptions and salicylic acid treatments

Treatments	Leaf water content (%)	Leaf temperature (˚C)	Membrane stability index (%)	Chlorophyll content index
Irrigation				
I_1	74.73 ± 0.60 a	17.17 ± 0.24 b	48.65 ± 0.43 a	18.15 ± 0.36 a
I_2	72.91 ± 0.92 a	19.17 ± 0.23 a	45.88 ± 0.71 b	16.63 ± 0.27 b
I_3	70.06 ± 0.63 b	19.02 ± 0.24 a	44.87 ± 0.72 b	15.41 ± 0.34 c
F test	15.60*	42.91**	12.81*	8.13*
Salicylic acid (mM)				
0	70.98 ± 0.81 a	18.84 ± 0.44 a	44.51 ± 0.87 c	16.01 ± 0.52 c
1	73.04 ± 1.26 a	18.72 ± 0.45 a	46.13 ± 0.91 bc	16.37 ± 0.47 bc
2	73.45 ± 0.95 a	18.36 ± 0.29 a	46.85 ± 0.73 ab	17.07 ± 0.53 ab
3	72.80 ± 1.08 a	17.90 ± 0.41 a	48.37 ± 0.65 a	17.47 ± 0.52 a
F test	1.26 ns	2.41 ns	7.33**	3.96*

*, **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

Different letters in each column indicate a significant difference at $p \leq 0.05$.

I_1, I_2 and I_3: normal irrigation, and irrigation disruptions from tassel emergence up to seed formation and from tassel emergence up to maturity, respectively

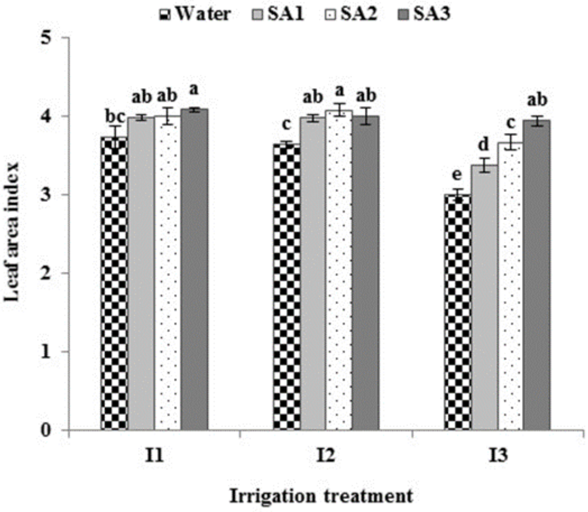

Figure 1. Effect of salicylic acid on leaf area index of maize under different irrigation treatments

Different letters indicate a significant difference at $p \leq 0.05$; I_1, I_2 and I_3: normal irrigation, irrigation disruptions from tassel emergence up to seed formation and from tassel emergence up to maturity, respectively; SA_1, SA_2 and SA_3: SA foliar application with concentrations of 1, 2 and 3 mM

negative. The highest positive correlation with grain, oil, and protein yields was recorded for LAI, followed by LWC and chlorophyll content index (Table 4).

Table 3. Means ± SE of grain yield and grain oil and protein percentages, and yields of maize plants affected by irrigation disruptions and salicylic acid treatments

Treatments	Grain yield (kg ha^{-1})	Oil percentage (%)	Oil yield (kg ha^{-1})	Protein percentage (%)	Protein yield (kg ha^{-1})
Irrigation					
I$_1$	10514.3 ± 345.1 a	5.92 ± 0.09 a	624.9 ± 26.87 a	6.08 ± 0.17 c	642 ± 32.97 a
I$_2$	9757.7 ± 326.7 b	6.04 ± 0.17 a	590.7 ± 29.35 a	6.55 ± 0.14 b	640.1 ± 28.17 a
I$_3$	6610.9 ± 261.2 c	5.15 ± 0.07 b	341.3 ± 15.97 b	7.46 ± 0.13 a	493.1 ± 20.57 b
F test	251.6**	11.37*	87.18**	14.48*	21.98**
Salicylic acid (mM)					
0	7687.6 ± 500.5 c	5.56 ± 0.16 a	431 ± 33.73 c	6.35 ± 0.23 a	480.8 ± 22.91 c
1	9080.5 ± 691.4 b	5.61 ± 0.19 a	515.6 ± 49.76 b	6.71 ± 0.27 a	597.3 ± 33.63 b
2	9114.5 ± 651.1 b	5.8 ± 0.21 a	538.4 ± 54.33 ab	6.82 ± 0.24 a	609.8 ± 27.3 b
3	9961.1 ± 694.6 a	5.84 ± 0.21 a	591 ± 55.98 a	6.91 ± 0.28 a	679.1 ± 41.45 a
F test	15.33**	0.77 ns	8.76**	1.85 ns	13.99**

*, **: Significant at p≤ 0.05 and p≤ 0.01, respectively

Different letters in each column indicate a significant difference at p≤ 0.05.

I$_1$, I$_2$ and I$_3$: normal irrigation, irrigation disruptions from tassel emergence up to seed formation and from tassel emergence up to maturity, respectively

Table 4. Correlations of coefficients between various traits of maize

	1	2	3	4	5	6	7	8	9	10
1- Leaf water content	1									
2- Leaf temperature	-0.678*	1								
3- Membrane stability index	0.782*	-0.698*	1							
4- Chlorophyll content index	0.784*	-0.781*	0.773*	1						
5- Leaf area index	0.789*	-0.377	0.656	0.679*	1					
6- Grain yield	0.900**	-0.508	0.742*	0.798**	0.911**	1				
7- Oil percentage	0.739*	-0.357	0.326	0.611	0.781*	0.842**	1			
8- Oil yield	0.883**	-0.485	0.644	0.773*	0.900**	0.987**	0.917**	1		
9- Protein percentage	-0.821**	0.658	-0.574	-0.849**	-0.729*	-0.897**	-0.888**	-0.929**	1	
10- Protein yield	0.813**	-0.278	0.739*	0.681*	0.932**	0.925**	0.685*	0.881**	-0.663	1

*, **: Significant at p≤ 0.05 and p≤ 0.01, respectively

Discussion

The decreased relative water content of leaves due to drought stress (Table 2) was related to reduced water uptake by roots and increased transpiration by the leaves, which ultimately leads to the closure of stomata and reduced photosynthesis (Agila *et al.* 2005). It has been reported that the decrease in the relative water content of the leaves in drought-stressed plants increases abscisic acid biosynthesis in leaves, which causes stomata closure and prevents water loss (Ghassemi *et al.* 2019). The closure of stomata reduces the cooling caused by transpiration and, therefore, enhances leaf temperature. Drought stress also increases the generation of reactive oxygen species (ROS), which damages cell membranes and enhances the leakage of electrolytes, leading to a low membrane stability index. Abdoli and Ghassemi-Golezani (2021) reported that SA enhances antioxidant capacity via increasing anthocyanins, phenolics, and flavonoid contents, thus improving ROS detoxification under stressful conditions. Application of SA enhances root growth in maize (Shao *et al.* 2018) and antioxidant enzymes activities in rapeseed (Ghassemi-Golezani *et al.* 2019), resulting in higher LWC and less LT and membrane damage under drought stress (Table 2). It is also reported that SA application enhances membrane stability under stressful conditions by decreasing lipid peroxidation and increasing leaf water content, proline, chlorophylls a and b, and carotenoids contents of *Mentha pulegium* (Farhadi and Ghassemi-Golezani 2021).

Degradation of chlorophyll by reactive oxygen species produced under drought stress is one of the most important reasons for chlorophyll depletion (Table 2). It has been reported that the reduction of photosynthetic pigments under drought stress is the result of chloroplast destruction, chlorophyll photooxidation, inhibition of chlorophyll synthesis, and activation of chlorophyllase (Banks 2018). Decreasing LAI under drought stress is the result of reduced cell elongation and division in maize plants (Ghassemi-Golezani *et al*. 2016a). The SA treatment increases photosynthetic pigments (Table 2) by improving the antioxidant capacity of cells and synthesizing new chlorophylls (Ghassemi-Golezani and Lotfi 2015), which promotes leaf growth (Figure 1). It has been reported that SA enhances photosynthesis via increasing LAI and the activity of the Rubisco enzyme (Shao *et al*. 2018).

Drought stress reduces assimilates through reducing chlorophyll content (Table 2) and leaf area (Figure 1), disturbing photosynthesis, transpiration, and metabolic processes and uptake and transport of nutrients (Nawaz *et al*. 2012), that causing a decline in grain yield (Table 3). The reduction of photosynthesis and limited flow of assimilates to grains under water stress leads to a loss of grain weight and yield (Saeidi *et al*, 2017). Under these conditions, the ratio of grain protein to oil increases, resulting in a decrease in oil percentage and an increase in grain protein percentage (Ghassemi-Golezani *et al*. 2016b).

Increasing grain yield by SA treatment (Table 3) was the result of improving leaf water content, chlorophyll content index (Table 2), and LAI (Figure 1), which had a direct effect on increasing oil and protein yields per unit area (Table 3). Bakry *et al.* (2012) also found that SA enhances the growth and grain yield of flax by improving source-sink relationships and photosynthetic activities. It seems that the treatment of plants with SA increases the leaf chlorophyll index (Table 2) and the leaf area index (Figure 1), leading to optimal use of solar radiation and ultimately to an increase in the rate of photosynthesis (Najafabadi and Ehsanzadeh 2017), grain yield (Table 3) and oil and protein yields per unit area (Table 3). Exogenous SA improves grain yield under drought stress by increasing the chlorophylls a and b and carotenoids, ground green cover and vegetative organs (leaves and stems), and flowers masses (Ghassemi-Golezani and Solhi-Khajemarjan 2021).

The highest positive and significant correlations of LAI, LWC, and chlorophyll content index with grain, oil, and protein yields (Table 4) suggest that a greater LAI, LWC, and chlorophyll content index can enhance the grain, oil, and protein yields of maize. Therefore, improving these traits via plant breeding or hormonal treatments could be an effective way to promote maize production under various levels of water availability.

Conclusion

Irrigation disruption at reproductive stages caused an increase in leaf temperature and protein percentage and a decrease in LWC, MSI, chlorophyll content, LAI, and grain, oil, and protein yields, compared to normal irrigation. Irrigation disruptions from tassel emergence up to seed formation and from tassel emergence up to maturity negatively affected the physiological traits and yield of maize. Thus, water supply during these growth stages is essential for improving maize yield. Exogenous SA increased mean LWC, MSI, chlorophyll content, LAI, and grain, oil, and protein yields of maize. Foliar spray of 3 mM SA in comparison with other concentrations showed a significant advantage in most of the traits, especially in grain, oil, and protein yields. Therefore, the application of SA is a useful way to mitigate the negative impacts of drought stress on maize.

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Conflict of interest

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

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بهبود عملکرد فیزیولوژیکی و محصول دانه ذرت با تیمار سالیسیلیک اسید تحت تنش خشکی

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چکیده

یک آزمایش مزرعه‌ای به‌صورت طرح کرت‌های خرد شده بر پایه بلوک‌های کامل تصادفی با سه تکرار در سال ۱۳۹۹ اجرا گردید تا اثر سطوح مختلف سالیسیلیک اسید (SA) بر صفات فیزیولوژیکی و محصول دانه، روغن و پروتئین ذرت (MV 524) مورد بررسی قرار گیرد. تیمارهای آبیاری شامل آبیاری معمول (آبیاری پس از ۶۰ میلی‌متر تبخیر) و قطع آبیاری از ظهور تاسل تا تشکیل دانه و از ظهور تاسل تا رسیدگی بودند. گیاهان با سه سطح SA (۱، ۲ و ۳ میلی‌مولار) و آب تیمار شدند. تیمارهای آبیاری و SA به ترتیب در کرت‌های اصلی و فرعی قرار گرفتند. قطع آبیاری در مراحل زایشی سبب کاهش میانگین محتوای آب برگ (LWC)، شاخص پایداری غشا (MSI)، شاخص کلروفیل، شاخص سطح برگ (LAI)، محصول دانه، درصد روغن، محصول روغن و پروتئین و افزایش دمای برگ و درصد پروتئین دانه در مقایسه با آبیاری معمول شد. کاربرد SA، به ویژه با غلظت ۳ میلی‌مولار، میانگین LWC، MSI، شاخص کلروفیل، شاخص سطح برگ و محصول دانه، روغن و پروتئین ذرت را افزایش داد. بیشترین همبستگی مثبت با محصول دانه، روغن و پروتئین مربوط به شاخص سطح برگ و پس از آن LWC و شاخص محتوای کلروفیل بود. این نتایج نشان می‌دهد که تأمین آب در مراحل زایشی برای تولید موفق ذرت ضروری است. با وجود این، محلول‌پاشی ۳ میلی مولار SA می‌تواند عملکرد مزرعه‌ای ذرت را تحت فراهمی معمول و محدود آب به‌طور قابل توجهی بهبود بخشد.

واژه‌های کلیدی: تنش خشکی؛ تنظیم کننده رشد؛ ذرت؛ روغن دانه؛ کلروفیل