Arab Universities Journal of Agricultural Sciences
(2025) 33 (1) 49-57
Website: http://ajs.journals.ekb.eg

Maize Yield and Quality as Affected by Salicylic or Ascorbic Acids under Irrigation Regimes

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https://doi.org/10.21608/AJS.2025.360885.1597

Received 16 February 2025; Accepted 5 May 2025

Keywords:

Cereals, Chlorophyll Content, Non-Enzymatic Antioxidant, Osmo-Protectants, Water Deficiency

Abstract: Appropriate methods should be implemented to address water scarcity in dry and semi-dry areas worldwide. Thus, the current study assessed the potential of salicylic (SA) and ascorbic (AsA) acids in enhancing maize tolerance to drought. In a strip-plots design, three irrigation regimes (70%, 85% and 100% of crop water requirements, designated as $I_{70\%}$, $I_{85\%}$ and $I_{100\%}$, respectively) and three anti-stress treatments (SA 150 mg L^{-1}, AsA 150 mg L^{-1}, and control treatment, CK) were applied in three replicates. Findings revealed that SA and AsA enhanced plant pigments under moderate or severe drought, surpassing the corresponding control. A reduction in proline concentration was observed under $I_{70\%}$ and $I_{85\%}$ with the application of SA or AsA. Both SA and AsA recorded the highest values of 100-grain weight and grain yield under severe drought. The most effective combinations for achieving the highest values were $I_{70\%}$ × SA (for crude protein percentage) and $I_{100\%}$ × SA or AsA (for carbohydrates percentage). It can be concluded that maize in arid climates can be treated with salicylic acid (150 mg L^{-1}) to achieve satisfactory yield and quality, especially during moderate deficit irrigation.

1 Introduction

Appropriate irrigation water enhances water absorption, mineral uptake, plant metabolism and development, cell number and division, photosynthesis, and fresh and dry matter production (Abdulnaser et al 2021). In contrast, water availability during drought reduced mineral absorption and photosynthesis, while increasing reactive oxygen species (ROS), leading to various damage in plant growth (Ibrahim et al 2019). ROS accumulation reduces light absorption, disrupts photosynthetic electron transport, and triggers lipid peroxidation, resulting in photo-oxidative damage to photosystems (Yudina et al 2020). Accordingly, reductions in growth traits such as plant height and fresh and dry weight**,** leaf number, stem diameter and leaf area were observed under drought (Farouk et al 2023).

Recently, the use of antioxidants, such as salicylic (SA) and ascorbic (AsA) acids, as an agronomic practice to lessen drought damage, has gained significant attention. In this context, SA plays a role in regulating numerous biochemical pathways in plants, including photosynthesis, proline and nitrogen metabolism, and the antioxidant defense system, while also diminishing the effects of abiotic stresses (EL-Hawary and Nashed 2019). As a non-enzymatic antioxidant, SA modulates various biochemical processes in plants, such as stomatal closure, ion uptake, and transpiration (Ramadan et al 2025). Moreover, SA boosts photosynthetic activity and enhances water absorption and ion transport (Klessig et al 2018). Applying SA on maize led to improvements in growth traits, such as plant height and leaf area index, by stimulating plant hormones like auxins and cytokinins, which promote cell expansion and division, while also reducing the adverse effects of abiotic stress (Abdulnaser et al 2021). The exogenous supply of SA significantly increased chlorophyll a, chlorophyll b, and carotenoids in maize compared to the control treatment (Ibrahim et al 2019).

Foliar application of ascorbic acid (AsA) in maize increased chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll compared to untreated plants (El-Hawary and Nashed 2019). AsA significantly reduces photo-oxidative stress by neutralizing ROS, preserving the integrity of chloroplast membranes and enhancing the photosynthetic rate in maize plants (Alamri et al 2018).

Based on the crucial physiological actions of SA and AsA, the current study was performed to test their potential for alleviating the damages of drought on maize plants.

2 Materials and Methods

2.1 Study area features

During the 2022 and 2023 seasons, two field trials were conducted at the Agricultural Production and Research Station, National Research Centre, El-Nubaria region, El Bahira Governorate, Egypt (30.0°31.0′N, 30.0°18.0′E; 21.0 m a.s.l.). The basal physico–chemical traits and water status parameters of the experimental soil are presented in **Table 1**. The study area is characterized by an arid climate with warm summers. The average values of minimum air temperature (21.4 and 21.8°C), maximum air temperature (35.5 and 35.4°C), relative humidity (53.7 and 53.3%), wind speed (0.79 and 74.0 m sec–1), solar radiation (21.8 and 23.8 MJ m–2 day–1), and evapotranspiration, ETo (6.36 and 6.61 mm day–1) in the first and second seasons, respectively.

Table 1. Basal physico–chemical traits and water status parameters of the experimental soil

Parameter	Unit	Value
Physical trait		
Coarse sand	(%)	16.9
Fine sand	(%)	74.9
Silt	(%)	4.5
Clay	(%)	3.7
Soil texture		Sand
Chemical trait		
Soil acidity, pH		8.10
Electrical conductivity, EC	(dS m^{-1})	0.41
Ca^{2+}	(meq L^{-1})	1.42
Mg^{2+}	(meq L^{-1})	0.34
K^{+}	(meq L^{-1})	0.25
Na^{+}	(meq L^{-1})	1.58
Cl^{-}	(meq L^{-1})	1.31
CO_3^{2-}	(meq L^{-1})	Not detected
HCO_3^{2-}	(meq L^{-1})	1.44
SO_4^{2-}	(meq L^{-1})	0.84
Bulk density	(gcm^{-3})	1.56
Moisture status		
Field capacity	(% on weight basis)	13.0
Wilting point	(% on weight basis)	4.3
Available water	(% on weight basis)	8.7

2.2 Treatments, design, and crop husbandry

Three irrigation regimes and three anti-stress treatments were replicated thrice using a strip plot design. Based on a ratio of crop water requirements (ETc), the three irrigation regimes (70%, 85% and 100% of ETc, abbreviated as $I_{70\%}$, $I_{85\%}$ and $I_{100\%}$, respectively) were applied. The anti-stress treatments included SA, AsA and the control treatment (CK, water spraying). The net area of the experimental plot was 14 m², comprising five ridges (4 m long and 0.7 m wide). Three to five grains of maize (cv. Giza Triple-Cross-368, yellow) were sown in hills (on June 3rd in the first season and May 15th in the second season), with a spacing of 25 cm on one side of the ridge. Seedlings were thinned 25 days after sowing (DAS) to secure one plant per hill. Based on previous studies for SA (Zhang et al 2015) and for AsA (Sharma et al 2019), a concentration of 150 mg L^{-1} for each was applied using a knapsack sprayer equipped with one nozzle and a spray solution of 470 L ha^{-1}. Due to the difficulty of dissolving SA in water, a stock solution containing 750 mg L^{-1} of SA was prepared by dissolving 750 mg of SA powder in 100 mL of ethanol (96%), then diluted to one liter with water.

Subsequently, the solution was further diluted to 150 mg L^{-1}. Tween-20 ($C_{58}H_{114}O_{26}$) was used as a wetting agent for all foliar treatments. Both SA and AsA solutions were sprayed in equal portions four times, at 30, 45, 60 and 75 DAS.

Irrigation water requirements were determined using the daily reference ETo specified by FAO through the FAO Penman–Monteith equation (Allen et al 1998) for the maize growth cycle. Crop evapotranspiration (ETc) of maize was computed (Doorenbos and Pruitt 1977). Consequently, the amount of irrigation water was calculated as illustrated by Keller and Bliesner (2001).

2.3 Assessments

2.3.1 Physiological traits

Samples of the upper fourth leaves were collected at 80 DAS to assess plant pigments and proline concentration. In this regard, chlorophyll a (Formula 1), chlorophyll b (Formula 2), and carotenoids (Formula 3) were estimated in the laboratory according to Wellburn (1994). The leaf samples (100 mg fresh weight) were ground and extracted with 85% methanol. Afterwards, the extracts were centrifuged at 8000 rpm for 10 minutes. The pigments were quantified by spectrophotometry (Edutec EEQ 9023) at wavelengths of 665, 646, and 470 nm, respectively.

The amount of free proline was assessed using the technique explained by Bates et al (1973). The extraction was measured with a UV-160A UV Visible Recording Spectrometer (Bausch and Lomb Analytical Systems Divisions, Rochester, New York, USA) at 520 nm.

Chlorophyll *a* concentration (mg g FW^{-1}) =
$12.64 * A_{665} - 2.99 * A_{646}$ ……………………(1)

Chlorophyll *b* concentration (mg g FW^{-1}) =
$23.26 * A_{646} - 5.6 * A_{665}$ …………………….. (2)

Carotenoids concentration (mg L^{-1}) =
$\frac{1000*A_{470} - 0.89*Chl\ a - 52.02*Chl\ b}{245}$ ……..………… (3)

2.3.2 Agronomic traits

At 110 DAS, maize plants were harvested to obtain ears. Five ears were used to measure ear length and diameter and 100-grain weight. Whole collected ears of the experimental plot were utilized to estimate grain yield ha^{-1}.

2.3.3 Grain chemical traits

Samples of dried maize grains from three replicates for each treatment were milled to fine powder. Then, constant samples of dried fine powder were used to determine crude protein and total carbohydrate percentages (AOAC 2012).

2.3.4 Data analysis

The combined results of the two seasons were statistically analyzed using a two–way analysis of variance (ANOVA). The software program Costat, Version 6.303-2004, was utilized according to the procedures outlined by Casella (2008). When the F–test indicated significant variation ($p<0.05$), the treatment means were distinguished using the least significant difference test (LSD 5%).

3 Results and Discussion

3.1 Plant pigments

The data presented in **Table 2** revealed that supplying maize with lower amounts of water (I70% and I85%) than the recommended irrigation water (I100%) led to a notable reduction in plant pigments. The I70% treatment resulted in reductions of 45.3%, 53.5%, and 60.0% for chlorophyll a, chlorophyll b, and carotenoids, respectively, compared to I100%. The corresponding decreases observed with I85% were 21.8%, 29.9%, and 20.0%, respectively. Drought is recognized as a major challenge to agricultural production. In this context, deficit irrigation is a widely applied practice to achieve reasonable crop yields while conserving water (Hadid et al 2023). However, the physiological status of plants is disrupted, leading to a decline in crop growth with reduced water supplies, especially during instances of severe drought (Abdo et al 2024). In this regard, the inhibitive impact of drought on plant pigment concentration is considered a significant aspect of oxidative stress, which is reflected in the oxidation of photosynthetic pigments and chlorophyll degradation (Doklega et al 2024). Under stress conditions, reactive oxygen species (ROS) are naturally produced (Mittler et al 2004), contributing to the induction of redox homeostasis and chlorophyll degradation (Ramadan et al 2023).

The foliar-applied SA and AsA significantly increased chlorophyll a, chlorophyll b, and carotenoids compared to the control treatment (**Table 2**). The increments amounted to 1.19 and 1.14 times for chlorophyll a, 1.20 and 1.70 times for chlorophyll b, and 1.15 and 1.11 times for carotenoids due to the application of SA and AsA, respectively. SA and AsA have demonstrated a significant role in coping with or mitigating drought injury. SA and AsA are considered anti-stress substances related to photosynthesis and nutrient uptake, thus promoting plant growth and development (Saudy et al 2023). SA potentially enhances plant tolerance by stimulating genes associated with stress responses and increasing levels of various sugars (Zhao et al 2021). Therefore, SA recorded the maximum values of plant pigments under well-watered conditions. Under moderate to severe drought, SA and AsA exhibited significant enhancements in the plant pigments of maize, surpassing the corresponding control treatment.

Table 2. Leaf pigments of maize as influenced by irrigation regime and anti-stress treatment

Treatment		**Chlorophyll *a* (mg g^{-1} F.W.)**	**Chlorophyll *b* (mg g^{-1} F.W.)**	**Carotenoids (mg g^{-1} F.W.)**
Irrigation				
$I_{70\%}$		1.63	0.87	0.32
$I_{85\%}$		2.33	1.31	0.64
$I_{100\%}$		2.98	1.87	0.8
Anti-stress (A)				
SA		2.48	1.44	0.62
AsA		2.38	1.40	0.60
CK		2.08	1.20	0.54
I×A				
$I_{70\%}$	SA	1.79	0.94	0.36
	AsA	1.71	0.91	0.34
	CK	1.40	0.75	0.27
$I_{85\%}$	SA	2.49	1.42	0.68
	AsA	2.38	1.39	0.66
	CK	2.11	1.11	0.58
$I_{100\%}$	SA	3.16	1.97	0.83
	AsA	3.03	1.91	0.82
	CK	2.74	1.74	0.76
LSD 5%				
I		0.06	0.04	0.02
A		0.05	0.01	0.01
I × A		0.09	0.03	0.01

$I_{70\%}$, $I_{85\%}$and $I_{100\%}$: irrigation by 70, 85 and 100% of crop water requirements respectively; SA: salicylic acid, AsA: ascorbic acid, CK: check treatment (tap water); F.W.: fresh weight.

3.2 Proline concentration

Data illustrated in **Fig 1** clarified that as drought increased, proline concentration rose. Thus, the $I_{70\%}$ treatment exhibited concentrations 1.54 and 3.78 times higher than those of $I_{85\%}$ and $I_{100\%}$, respectively. Proline largely accumulates in plants exposed to adverse ecological conditions (Helal et al 2024). Furthermore, proline enhances antioxidant enzymes, thereby reducing oxidative stress damage and preserving cellular bio-constituents (Majumder et al 2009). Conversely, the application of SA and AsA decreased proline concentration by approximately 19.5% and 16.3%, respectively, compared to the control treatment. Regarding the interaction, the lowest proline value was obtained with SA or AsA × $I_{100\%}$. Additionally, reductions in proline concentration were observed under $I_{70\%}$ and $I_{85\%}$ with the application of SA or AsA. Moreover, SA application promoted enzymatic oxidative protection and proline formation, thus minimizing excess ROS formation and alleviating the negative impacts of stress (Zhang et al 2015).

3.3 Yield traits

As anticipated, full irrigation ($I_{100\%}$) yielded the highest values for ear traits, 100-grain weight, and grain yield of maize (**Table 3**). With I100% application, the increases in ear length, ear diameter, 100-grain weight, and grain yield were 2.06 and 1.46 times for ear length, 1.22 and 1.12 times for ear diameter, 1.38 and 1.26 times for 100-grain weight, and 2.00 and 1.45 times for grain yield, compared to $I_{70\%}$ and $I_{85\%}$, respectively. Suppressing maize growth under limited water may be attributed to reduced cell division and extension (Jaleel et al 2009). Drought reduced ear length and diameter, ear grain number, ear grain weight, and grain yield (Salem et al 2021).

SA increased ear length, ear diameter, 100-grain weight, and grain yield by approximately 22.4%, 9.1%, 14.3%, and 34.5%, respectively, compared to the control treatment. The increases in ear length, ear diameter, 100-grain weight, and grain yield due to the application of AsA were 18.6%, 8.4%, 11.6%, and 22.0%, respectively, in relation to the control treatment. As a non-enzymatic antioxidant, SA serves as an effective defensive blocker for plants against oxidative stresses (Sharma et al 2019). Ascorbate peroxidase is the key enzyme in the ascorbate–glutathione pathway; AsA plays an essential role in protecting against oxidative damage by eliminating various free radicals (Bilska et al 2019). Exogenous application of AsA stimulated the physiological and biochemical features, productivity, and water utilization of drought-stressed sunflower (Saudy et al 2021). AsA helps preserve plant tissues from the effects of abiotic stresses by reducing ROS, thus enhancing both crop yield and quality (Ali et al 2024).

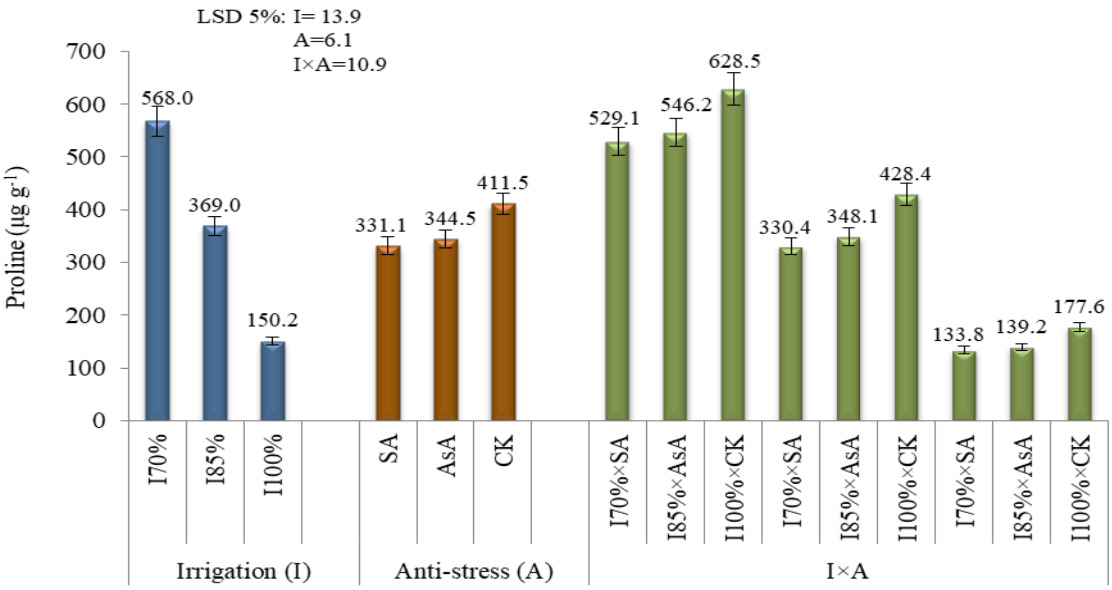

Fig 1. Leaf proline concentration (based on fresh weight) of maize as influenced by irrigation regime and anti-stress treatment. $I_{70\%}$, $I_{85\%}$ and $I_{100\%}$: irrigation by 70%, 85% and 100% of crop water requirements, respectively; SA: salicylic acid, AsA: ascorbic acid, CK: check treatment (tap water)

Table 3. Maize yield traits as influenced by irrigation regime and anti-stress treatment

Treatment		Ear length (cm)	Ear diameter (cm)	100-grain weight (g)	Grain yield (kg ha^{-1})
Irrigation (I)					
$I_{70\%}$		16.95	2.97	25.04	3084.36
$I_{85\%}$		23.81	3.22	27.46	4259.22
$I_{100\%}$		34.94	3.63	34.60	6193.72
Anti-stress (A)					
SA		27.17	3.37	30.54	5106.54
AsA		26.33	3.35	29.83	4632.78
CK		22.20	3.09	26.73	3797.98
I×A					
$I_{70\%}$	SA	17.15	3.08	26.08	3630.95
	AsA	17.38	2.99	25.78	3238.46
	CK	16.31	2.84	23.27	2383.66
$I_{85\%}$	SA	26.63	3.27	28.15	4740.60
	AsA	25.30	3.30	27.80	4459.66
	CK	19.50	3.09	26.42	3577.38
$I_{100\%}$	SA	37.72	3.78	37.40	6948.05
	AsA	36.32	3.75	35.92	6200.22
	CK	30.78	3.35	30.49	5432.90
LSD 5%					
I		1.87	0.23	1.22	295.13
A		0.74	0.11	0.96	317.53
I × A		1.56	0.22	1.75	745.08

$I_{70\%}$, $I_{85\%}$ and $I_{100\%}$: irrigation by 70%, 85% and 100% of crop water requirements respectively; SA: salicylic acid, AsA: ascorbic acid, CK: check treatment (tap water).

Under well-watered conditions, both SA and AsA exceeded the control treatment in achieving the maximum values for all maize yield traits. The differences between SA and AsA were not significant for ear length, ear diameter, and 100-grain weight. The grain yield of the SA treatment was greater than that of the AsA treatment. Under severe drought, the SA treatment recorded the highest values for ear diameter and 100-grain weight and grain yield, significantly equaling AsA for both 100-grain weight and grain yield. Both SA and AsA exhibited similar improvements in ear length and grain yield under moderate drought.

3.4 Grain chemical composition

As irrigation water increases, the percentage of crude protein **(Fig 2)** decreases, while the percentage of carbohydrates **(Fig 3)** increases. Notably, I70% recorded the highest crude protein percentage and the lowest carbohydrate percentage in maize grains. Furthermore, drought negatively impacts maize grain quality by reducing nutrient availability, uptake, and metabolism (Mubarak et al 2021). Since drought can adversely affect soil physico-chemical traits, particularly moisture status, it may impede the movement and uptake of nutrients (Amtmann and Blatt 2009). As plants encounter nutrient deficiencies, dry matter accumulation and grain quality decline (Ramadan et al 2024).

Both SA and AsA surpassed the control treatment in improving crude protein (1.20 and 1.10 folds) and carbohydrates (1.07 and 1.06 folds), respectively. However, SA outperformed AsA in crude protein by approximately 1.10 folds. $I_{70\%}$ × SA (for crude protein percentage) and $I_{100\%}$ × SA or AsA (for carbohydrates percentage) were the effective combinations for recording the maximum values. Furthermore, SA can potentially improve mineral uptake while regulating ionic balance, thus mitigating the severe impacts of abiotic stresses (Santisree et al 2020). As mentioned previously, the SA application enhanced the formation of proline (Zhang et al 2015). In this context, it has been reported that proline increased sugar levels and stimulated nitrogen metabolism, leading to higher yield quality (Takeuchi et al 2008). This could explain the increase in maize grain protein under drought conditions. Under harsh conditions, proline helped maintain plant cell health by improving the stability of proteins and cellular structures (Szabados and Savouré 2010). Proline also has the potential to modify ion levels within cells subjected to abiotic stress, thereby improving growth (Hadid et al 2024).

Based on the significant physiological roles of SA and AsA in mitigating eco-stresses, maize pigments, growth and grain yield were improved under mild and severe drought. However, the beneficial effects of SA were more pronounced and greater than those of AsA in this regard.

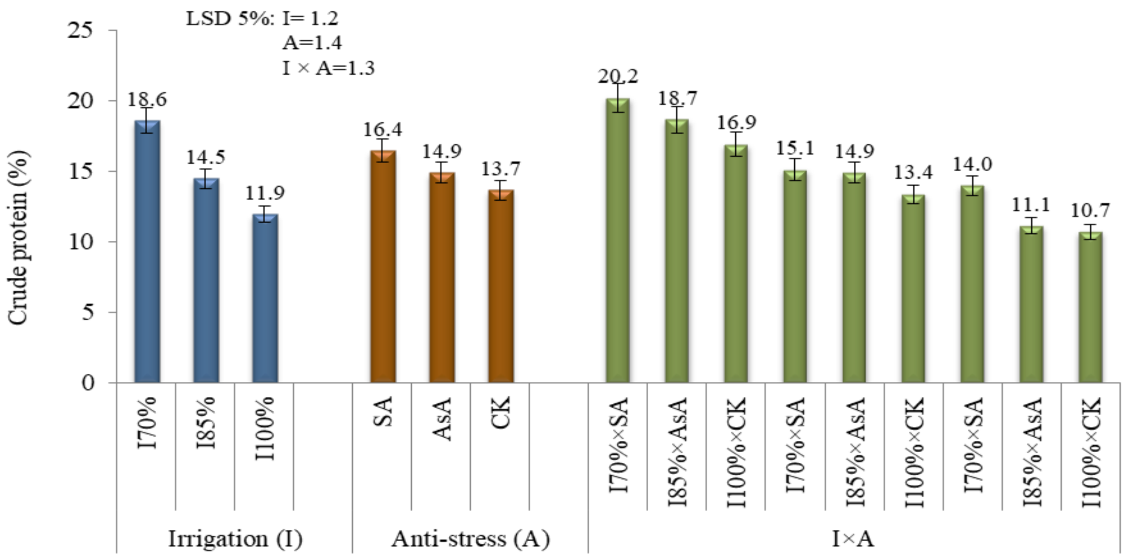

Fig 2. Grain crude protein percentage of maize as influenced by irrigation regime and anti-stress treatment. $I_{70\%}$, $I_{85\%}$ and $I_{100\%}$: irrigation by 70%, 85% and 100% of crop water requirements, respectively; SA: salicylic acid, AsA: ascorbic acid, CK: check treatment (tap water)

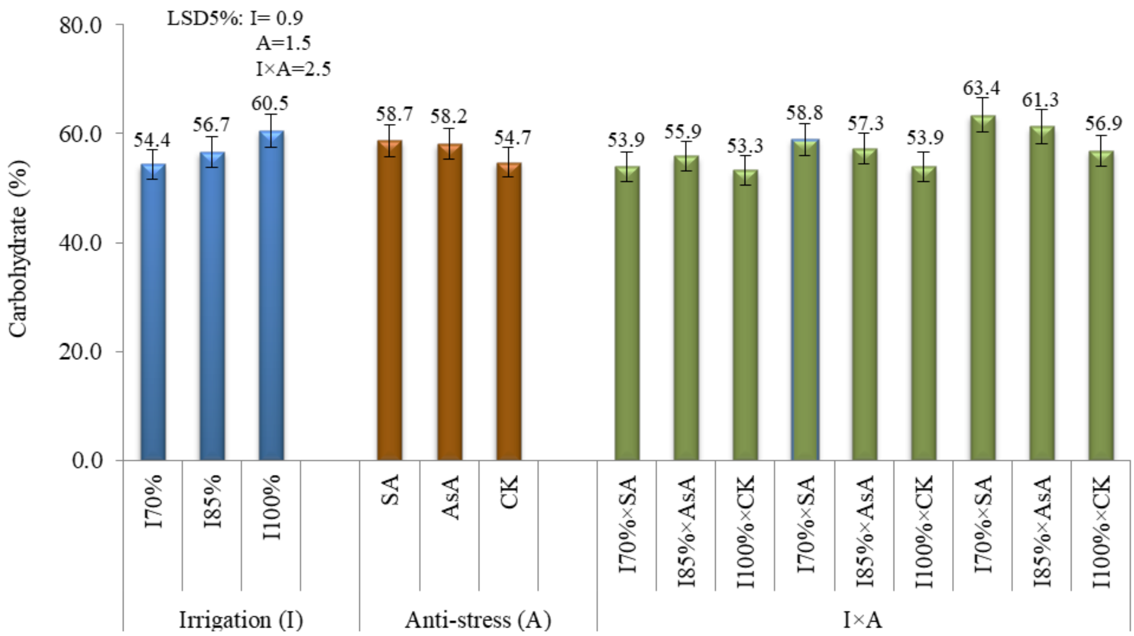

Fig 3. Irrigation regimes and anti-stress treatments influence the grain carbohydrate percentage of maize. $I_{70\%}$, $I_{85\%}$ and $I_{100\%}$: irrigation by 70%, 85% and 100% of crop water requirements, respectively; SA: salicylic acid, AsA: ascorbic acid, CK: check treatment (tap water)

4 Conclusion

Despite both salicylic and ascorbic acid treatments having a favorable role in mitigating the drought effects on maize plants compared to untreated ones, the application of salicylic acid was more effective in enhancing growth and yield. Under deficit irrigation, particularly during moderate drought, maize growers are advised to treat plants with salicylic acid (150 mg L^{-1}) to achieve acceptable productivity and quality.

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