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SALICYLIC ACID FOLIAR SPRAY PROMOTES YIELD, YIELD COMPONENTS, AND PHYSIOLOGICAL CHARACTERISTICS IN FOXTAIL MILLET UNDER DROUGHT STRESS

MOHAMMAD-ALI KARIMIAN[1], BAHMAN FAZELI-NASAB[1*], R. Z. SAYYED[2,*], NOSHIN ILYAS[3], WALEED HASSAN ALMALKI[4], SIDDHARTH VATS[5], SHAHID MUNIR[6], HINA SAIF[7] AND ASHFAQ AHMAD RAHI[6]

[1]*Department of Agronomy and Plant Breeding, Agriculture Institute, Research Institute of Zabol, Zabol, 9861335856, Iran*
[2]*Department of Microbiology, PSGVP Mandal's S I Patil Arts, G B Patel Science, and STKV Sangh Commerce College, Shahada, 425 409, India*
[3]*Department of Botany, PMAS Arid Agricultural University, Rawalpindi, 46300, Pakistan*
[4]*Department of Pharmacology, College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia*
[5]*School of engineering and technology, Noida International University, Gautam Buddha Nagar*
[6]*Pesticide Quality Control Laboratory, Multan, 60000 Punjab, Pakistan*
[7]*Department of Environmental Sciences, The Women University Multan, Punjab, Pakistan*
[*]*Corresponding author's email: bfazeli@uoz.ac.ir; sayyedrz@gmail.com*

Abstract

Drought is one of the major factors affecting plants' growth and development. , The application of plant hormones like salicylic acid (SA) is known to increase a crop's resistance to drought stress (DS) and help plants grow under drought conditions. Therefore, the present study aimed to evaluate the effect of salicylic acid foliar spray (SAFS) on yield and yield components of foxtail millet under different levels of DS. The present study reports the effect of SA foliar spray on yield and its components in foxtail millet Basten cultivar under drought stress conditions with three irrigation levels (45%, 65%, and 85% humidity of field capacity) as the main factor and four SA levels (0 to 2 mM) as the subplot. The results revealed that the fresh forage yield (36.76 ton/ha) and plant height (89.6 cm) were obtained from control and three mM SAFS treatments. The highest stem yield (6.971 ton/ha), leaf yield (4.947 ton/ha), grain yield (2.568 ton/ha), panicle length (18.7 cm), seed number per panicle (3670), 1000-seed weight (3.64 g) and several leaves per plant (11.43) were obtained by foliar spraying of 1.0 mM SA under normal conditions. The maximum harvest index (61.66%) was obtained under moderate stress conditions and 1.0 mM SA foliar spray. The highest levels of chlorophyll a, b, and total chlorophyll were 6.35, 4.02, and 10.37 mg/WW, respectively, from the treatment without drought stress and the application of 1.0 mM SA. The results showed that spraying 1.0 and 3 mM SA under stress and normal conditions improved yield components in foxtail millet Basten cultivar in Sistan weather conditions.

Key words: Foxtail millet; Harvest index; Hormone; Leaf yield; Low irrigation.

Introduction

Millets, a group of cereals belonging to the Graminae family, have been cultivated worldwide as a food and forage. Pearl millet, Foxtail millet (*Setaria italica* L. Beauv), Common millets, and Finger millet are the most important species of millets. Foxtail millet is commonly planted annually for human consumption. This plant is an important food and fodder crop suitable for uncultivated, marginal, and arid land areas (Niu *et al*., 2018). This plant is a broadly planted dryland vegetable with higher drought endurance and water Use efficiency (WUE) than other plants such as maize, milo-maize, and *Triticum aestivum* (Lata *et al*., 2013). The plant possesses good tolerance to drought and salinity stress.

The growth and development of crops are constantly affected by various environmental factors (Kannepalli *et al*., 2021; Ilyas *et al*., 2020; Sagar *et al*., 2022; Nasab *et al*., 2021). Water scarcity is one of the most crucial abiotic stresses for plant growth and the most common environmental stress worldwide (Khan *et al*., 2021; Fallah *et al*., 2021; Najafi *et al*., 2021). Water is known to cause survival limitations in arid and semi-arid regions (Gupta *et al*., 2020; Khan *et al*., 2020).

CO_2 restriction due to the closure of pores resulting from drought-induced pressure loss causes reduced photosynthetic enzyme activity and biochemical components associated with phosphate triose formation. The reduced CO_2 level is one of the main limiting components of photosynthesis (Pandey & Shukla, 2015).

Drought stress (DS) reduces grain yield in three millet species, including foxtail, common, and pearl, mainly due to the reduced number of panicles per square meter and grains per cluster. Water stress reduced millet clusters' seed yield and grain number (Maqsood & Ali, 2007). It also negatively impacts the millet harvest index by decreasing seed number per cluster and plant, thus affecting the biomass yield of nutrifeed (Seghatoleslami *et al*., 2008).

Applying plant growth regulators such as SA and jasmonic acid improves the plant's resistance to abiotic stresses (Simaei *et al*., 2011). SA is a phenolic compound. It is an essential signal molecule in regulating a plant's response to abiotic stresses (Simaei *et al*., 2011). SA significantly reduces ionic leakage and toxic ion accumulation in plants, decreasing the effect of environmental stresses by increasing growth-regulating hormones such as auxins and cytokinins. Exogenous application of SA improves seed germination, photosynthesis, and growth parameters in mustard

(*Brassica juncea* L.), tomato, and height in wheat (Hayat *et al*., 2005; Habibi, 2012). and corn in water deficit conditions (Mehrabiyan *et al*., 2011). Cycocel and SA spray under optimal and water stress conditions increased spike length, panicle weight, grain weight per panicle, and grain yield of Shahriyar wheat (Jiriaie *et al*., 2009). Under DS conditions, SA treatment causes stomatal closure and maintained turgor pressure, resulting in cell elongation, cell enlargement, and plant growth (Khodary, 2004). Therefore, the present study aimed to evaluate the effect of SAFS on yield and yield components of foxtail millet under different levels of DS.

Materials and Methods

Study site: The research was conducted at the University of Zabol (latitude 30º 54' N, longitude 61º 41' E) as a split plot based on an RCBD with three replicates. Before the experiment, soil type, physical properties, and soil chemicals were determined (Table 1).

Planting: The dimensions of the main plots and subplots were 3×14 m and 3×3 m, respectively, with 50 cm spacing between cultivating rows, 6 cm spacing within the cultivating rows, 2 m spacing between the replicates, and 1 m spacing between the main plots, and 50 cm spacing between subplots. Each subplot included six rows of planting (Fig. 1). The density was about 333000 plants per hectare.

Treatments: DS was the primary test factor (45%, 65%, and 85% humidity of the field capacity), with four levels of SA treatment as subplots (0, 0.75, 1.5, and 3 mM). Based on soil analysis results, a combination of chemical fertilizers, including triple superphosphate (100 $kg.ha^{-1}$, before planting), potassium sulfate (150 $kg.ha^{-1}$, before planting), and ammonium sulfate (75 $kg.ha^{-1}$ before planting and 75 $kg.ha^{-1}$ at stem elongation stage) was applied. Planting was carried out following seed disinfection with Tiram fungicide (2:1000).

Irrigation was at three days intervals until the plant was completely deployed. Volumetric water contents of field capacity and wilting point were 28.5% and 11.5%, respectively. The difference between the moisture of field capacity and the wilting point was considered as the available moisture. Volumetric water content was determined daily, and the irrigation time of different treatments was obtained. A tanker irrigated each plot after reaching 45%, 65%, and 85%. The soil moisture was measured using a Delta-T Devices Ltd UK TDR humidity meter. Hand weeding was carried out at 3-leaf to 4-leaf stages (Karimi *et al*., 2016).

Measurement of traits: Several traits, including fresh forage yield, stem yield, leaf yield, height, panicle length, grain yield, seed number per panicle, 1000- seed weight, and several leaves per plant and harvest index, were measured. Sampling was carried out in a 1 m^2 plot area from two middle rows during millet flowering time (June) after removing the marginal effect. Samples were transferred to the laboratory, where fresh forage weight was immediately measured using an A and D scale (Japan) with a precision rate of 0.01 g. Then samples were kept in the oven at the temperature of 74°C for 48 h to get dried. Leaf and stem weights were measured separately, and their yield was presented as ton/ha. Ten plants were randomly selected from each plot during seed maturation. Plant traits, such as height (using a meter), panicle length (using a digital caliper), number of leaves per plant, number of seeds per panicle, 1000 seed weight, and seed yield, were measured. The harvest index was calculated as follows:

The ratio of economic yield = Biological yield x 100

Table 1. Physico-chemical analysis of the site experiment soil*.

Soil texture	pH	EC (dS/m^{-1})	organic matter (%)	Minerals ($mg.kg^{-1}$)				
				N	**P**	**K**	**Mn**	**Cu**
Sandy loam	7.8	1.7	0.85	0.14	8.53	210.25	5.18	1.13
				Zn	Fe	Mg	Ca	Zn
				0.75	5.91	5/73	0/24	0.75

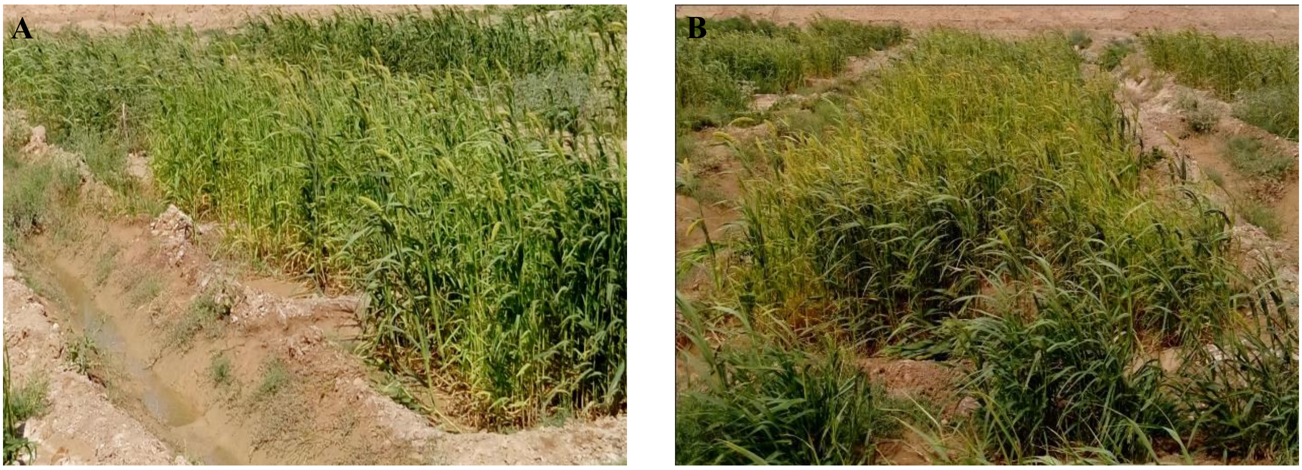

Fig. 1. The experiment farm of *Setaria italica* L. that was conducted as a split-plot, based on a randomized complete block design (A and B).

Measurement of photosynthetic pigments: Chlorophyll was measured using the Lichtenthal method. An 80% acetone was used as a control for calibration. A 100 mg of fresh leaves of the plant in porcelain mortar containing 15 ml of 80% acetone were ground, filtered, and the chlorophyll a and chlorophyll b contents were read at 663.2 and 646.8 nm, respectively.

Statistical analysis

Data analysis was performed using MSTATC software that involved the mean based on the Duncan multi-range test at a 5% probability level.

Results

Fresh forage yield: Analysis of variance (ANOVA) results showed that DS, SA, and their interactions at a 1% probability level significantly affect fresh forage yield (Table 2). The mean comparison of interactions between DS and SA treatment revealed the highest fresh forage yield (36.76 ton/ha) for control along with 2.0 mM SA treatment and the lowest fresh forage yield (17.89 ton/ha) under severe stress conditions and SA-free treatment (Table 3).

Stem yield: Variance analysis results revealed that DS, SA treatment, and their interaction significantly affect stem yield (Table 2). The mean Comparison of interactions between DS and SA treatment showed the highest stem yield (6.971 ton/ha) in control 1.0 mM SA application and the lowest stem yield (2.8 ton/ha) under severe stress conditions and SA-free treatment (Table 3).

Leaf yield: Variance analysis results (Table 2) showed that the effect of DS, SA application, and their interaction on leaf yield is significant at a 1% probability level. The mean Comparison of interactions between DS and SA treatment showed the highest leaf yield (4.947 ton/ha) in control × 1.0 mM SA application and the lowest leaf yield (1.101 ton/ha) under severe stress conditions, with no SA treatment (Table 3).

Plant height: DS, SA treatment, and their interactions significantly impacted plant height (Table 2). The mean Comparison of interactions between DS and SA treatment showed the highest plant height (89.6 cm) in control × 2.0 mM SA application and the lowest plant height (42.5 cm) in severe stress and SA-free treatment (Table 3).

Panicle length: DS, SA treatment, and their interactions significantly affected panicle length (Table 2). The mean Comparison of interactions of DS and SA treatment showed maximum panicle length (18.7 cm) in control x 1.0 mM SA treatment and minimum panicle length (3 cm) in severe stress conditions and SA-free treatment (Table 3).

Seed yield: The results revealed that DS, SA treatment, and their interaction on grain yield are significant at a 1% probability level (Table 2). Mean comparison results showed the highest grain yield (2.568 ton/ha) in control × 1.0 mM SA application and the lowest grain yield (0.301 ton/ha) under severe stress conditions and SA-free application (Table 3).

Number of seeds per panicle: Our results on the effect of DS, SA treatment, and their interactions on the number of seeds per panicle were significant at a 1% probability level (Table 2). The mean comparison of DS and SA treatment showed the highest seeds number per panicle (3670) in control and 1.0 mM SA treatment and the lowest seeds number per panicle (1051) under severe stress and SA-free treatment (Table 3).

1000-seed weight: DS, SA treatment, and their interactions on 1000-seed weight were significant at a 1% probability level (Table 2). The mean comparison of interactions of DS and SA showed the highest 1000-seed weight (3.64 g) in the control × 1.0 mM SA treatment and the lowest 1000-seed weight (1.09 g) under DS and SA-free treatment (Table 3).

Number of leaves per plant: Our results showed that DS, SA, and their interaction with leaf number per plant are statistically significant (Table 2). The mean comparison of interactions between DS and SA showed the highest number of leaves per plant (11.43) in control and 1.0 mM SA application and the lowest number of leaves per plant (8.11) in DS and SA-free treatment (Table 3).

Harvest index: Harvest index was severely affected by DS, SA treatment, and their interactions (Table 2). The mean comparison of interactions between DS and SA treatment showed the highest harvest index (61.66%) in medium stress and 1.0 mM SA treatment and the lowest harvest index (6.32%) in severe stress conditions and three mM SA treatment (Table 3). ANOVA revealed a significant impact of DS, SA, and their interaction on chlorophyll-a ($p<0.01$) (Table 4). The mean comparison of DS interaction with SA showed that the highest chlorophyll-a (6.35 mg / g fresh weight) was obtained from drought stress-free treatment and application of 1.0 mM SA. The lowest amount (1.51 mg / g fresh weight) was obtained under severe stress and lack of foliar application of SA (Table 5).

According to the analysis of the variance of the data (Table 4), DS, SA, and their interaction with chlorophyll b were significant($p<0.01$). Comparing the mean interaction effect of DS and SA showed that the highest amount of chlorophyll b (4.02 mg/g fresh weight) was obtained from treatment without DS and application of 1.0 mM SA. The lowest amount (0.24 mg / g fresh weight) was obtained under severe stress and lack of foliar application of SA (Table 5).

DS, SA, and their interaction on total chlorophyll were significant ($p<0.01$) (Table 4). Comparison of means, the interaction of DS and SA, showed that the highest total chlorophyll (10.37 mg/g fresh weight) was obtained from treatment without DS and application of 1.0 mM SA. The lowest amount (1.75 mg/g fresh weight) was obtained under severe stress and lack of foliar application of SA (Table 5).

Table 2. Effect of quantitative characteristics forages of foxtail millet Basten cultivar.

Source of variations	df	Fresh forage yield (ton/ha)	Stem yield (ton/ha)	Leaf yield (ton/ha)	Plant height (cm)	Panicle length (cm)	Seed yield (ton/ha)	No. of seeds per panicle	1000-Seed weight (g)	Number of leaves per plant	Harvest index
Replication	2	4.710^{ns}	0.15^{ns}	0.02^{ns}	1.863^{ns}	1.299^{ns}	0.007^{ns}	56405.028^{ns}	0.01^{ns}	0.211^{ns}	12.048^{ns}
DS (A)	2	45.672^{**}	3.482^{**}	2.953^{**}	31.706^{*}	63.734^{**}	3.586^{**}	5532648.528^{**}	3.9^{**}	1.229^{**}	361.706^{**}
Error (a)	4	4.713	0.278	0.058	8.683	1.343	0.046	23746.403	0.027	0.237	4.403
SA (B)	2	34.848^{**}	4.101^{**}	3.652^{**}	751.164^{**}	6.458^{**}	3.272^{**}	3018748.398^{**}	3.402^{**}	6.328^{**}	1646.577^{**}
A×B	6	73.957^{**}	3.098^{**}	1.120^{**}	454.804^{**}	13.958^{**}	1.018^{**}	1303671.787^{**}	0.949^{**}	1.104^{**}	664.341^{**}
Error (b)	18	2.995	0.059	0.025	4.761	0.342	0.011	14327.574	0.016	0.067	4.097
CV (%)	-	6.09	4.87	8.64	3.24	7.3	7.73	5.05	5.26	2.64	8.3

ns, *and **: are non-significant and significant at 5 and 1 probability levels, respectively

Table 3. Effect of interaction affects DS and SA on physiological characteristics of foxtail millet Basten cultivar.

DS	SA (mM)	Fresh forage yield (ton/ha)	Stem yield (ton/ha)	Leaf yield (ton/ha)	Plant height (cm)	Length of panicle (cm)	Seed yield (ton/ha)	No. of seeds per panicle	1000-Seed weight	Number of leaves/plant	Harvest index
85% Field capacity	Control	23.22f	3.491hi	1.346fg	55.5g	6.5f	0.887h	1741g	1.39f	8.65gh	14.92fg
	0. 5	25.41def	4.271f	2.409e	67.44e	11.2d	1.369f	2292ef	2.2e	9.37ef	24.96e
	1.0	30.66bc	6.971a	4.947a	78.9c	18.7a	2.568a	3670a	3.64a	11.43a	49.47b
	2.0	36.76a	5.749c	3.541c	89.6a	16.46bc	2.269c	3100c	3.174b	10.42c	8.34ij
65% Field capacity	00	19.97g	3.102ij	1.125g	47.64h	4.25g	0.541i	1369h	1.23fg	8.29hi	18.18f
	0. 5	24.74ef	4efg	1.667f	62f	10.1de	1.221fg	2241f	2.012e	9.12f	28.55d
	1.0	27.97cd	6.545b	4.531b	74.4d	17.63ab	2.47ab	2749d	3.25b	10.98b	61.66a
	2.0	33.43b	5.2d	3.133cd	85.54b	16.13bc	1.946d	3471ab	2.864c	9.96d	10.28hi
45% Field capacity	00	17.89g	2.8j	1.101g	42.5i	3g	0.301j	1051i	1.09g	8.11i	12.44gh
	0.5	23.41f	3.747gh	1.54fg	58.24g	8.3ef	1.069g	2095f	2e	8.99fg	22.1e
	1.0	26.68de	6.112c	4.125b	71.94d	17.33ab	2.312bc	2492e	3.177b	10.86bc	35.41c
	2.0	31.72b	4.755e	2.757de	81.34c	14.46c	1.728e	3297bc	2.59d	9.75de	6.32j

Different letters indicate significant differences based on Duncan's multiple range test (α=0.05)

Table 4. ANOVA of physiological characteristics of foxtail millet Basten cultivar.

Source of variations	df	Chlorophyll a	Chlorophyll b	Total chlorophyll
Replication	2	0.028^{ns}	0.017^{ns}	0.070^{ns}
DS (A)	2	4.142^{**}	4.096^{**}	12.606^{**}
Error (a)	4	0.011	0.131	0.141
SA (B)	3	17.835^{**}	9.835^{**}	49.864^{**}
A×B	6	4.850^{**}	4.883^{**}	16.891^{**}
Error (b)	18	0.096	0.050	0.045
CV (%)	-	7.22	10.07	3.28

ns, *, and ** are non-significant and significant at 5 and 1 probability levels, respectively

Table 5. Effects of interactions of DS and SA on physiological characteristics of foxtail millet Basten cultivar.

DS	SA (mM)	Chlorophyll a (mg/g^{-1}.fw)	Chlorophyll b (mg/g^{-1}.fw)	Total chlorophyll (mg/g^{-1}.fw)
85% Field capacity	00	2.31f	0.55f	2.86h
	0. 5	5.12c	2.42d	7.54e
	1.0	6.35a	4.02a	10.37a
	2.0	5.41bc	3.2c	8.61cd
65% Field capacity	00	1.94fg	0.27f	2.21i
	0.5	3.91d	1.82e	5.73f
	1.0	5.79b	3.67ab	9.46b
	2.0	5.3bc	3.17c	8.47cd
45% Field capacity	00	1.51g	0.24f	1.75j
	0.5	3.17e	0.62f	3.79g
	1.0	5.42bc	3.41bc	8.83c
	2.0	5.27bc	3.02c	8.29d

Different letters indicate significant differences at α=0.05

Discussion

DS is one of the detrimental negative impacts on plant yield. CO_2 and closure stomatal are the first responses to DS in the plant (of course, at first leaves sections), consequently decreasing photosynthetic activity (Hepworth *et al*., 2015).

The seedlings that lack water stress have notable morphological traits (El-Sabagh *et al*., 2017). The reduction in growth characteristics of Foxtail millet Bastan cultivar plants under DS conditions agrees with the results of (El-Sabagh *et al*., 2017) in various plants. Severe drought affects the percentage of leaf weight due to the shortening of internodes and decreasing the number of stems per plant (Akhondi & Safaarnejad, 2004). Some mechanisms like osmotic adjustment, protective proteins accumulated, and antioxidant materials' defense systems help plants tolerate stress conditions (Gürel *et al*., 2016).

DS reduces the leaf area index so that less water remains inside the cells, and reduced cell volume decreases weight (Haghshenas *et al*., 2020). The effect of drought and nitrogen fertilizer limitations on the above-ground part of forage pearl millet (*Pennisetum glaucum*) showed a reduction in dry leaf weight and stem wet and dry yields (Zabet *et al*., 2014). Plants with height lowered in more DS conditions are more sensitive to DS, so plant height can be used as a response type to drought and a criterion to detect and select tolerant genotypes for dry environmental conditions (Zou *et al*., 2007). DS probability reduces the number, Relative water content, cell division, and photosynthesis, and these factors affect the yield components (El-Esawi *et al*., 2018; Gurel *et al*., 2016).

The number of seeds and the weight of 1000 grains will usually decrease after being water-deficient. These factors also were related to the seed yield of foxtail millet. It is indicated that the irrigation disruption from the beginning of the flowering period reduces the number of seeds per panicle (Khomari *et al*., 2008). Water deficit stress diminished pod length in the major and secondary branches of cowpea, while the maximum pod length was observed in full irrigation treatment (Pakmehr *et al*., 2011).

SA is an effective signaling molecule that regulates plant tolerance to plant stresses (Wang *et al*., 2010). It regulates plants' physiological and biochemical properties and plant growth, and fruit yield, in plants (Liu *et al*., 2015). SA is a vital hormone for chlorophyll content (Fariduddin *et al*., 2003), carotenoid composition (Gao *et al*., 2012), and stomatal closure (Khokon *et al*., 2011).

SA increases the abscisic acid content, which causes more accumulation of proline, an amino acid required for the plant's defense system against stress. Accumulation of proline in cells is often observed under drought-stress conditions. Additionally, SA can improve nonenzymatic antioxidant and enzymatic activity like CAT, POX, and PPO. It also plays a central role in enhancing plant growth under drought stress by increasing plant tolerance to stress conditions and decreasing oxidative stress (Mutlu *et al*., 2016). It is indicated that the SA has significantly reduced the effects of salinity on the morphological traits by increasing the branch numbers, plant height, FW, and DW (El-Esawi *et al*., 2017).

Spraying SA increased biomass in soybean (Eraslan *et al*., 2007), which seems to be due to the antioxidant activity of this compound in the cell membrane. SA treatment improves the lignin content in the cell wall, which can be a critical factor in increasing plant biomass under DS conditions (Vafabakhsh *et al*., 2008). Foliar spraying of SA in corn increased leaf area, leaf number, height, plant dry weight, and root (Khodary, 2004). SA probably improves nutrient absorption under DS and salinity, increasing the plant height and growth rate (Eraslan *et al*., 2007). These effects of SA may be due to the more significant role of SA in water storage in plant cells and the increase in enzymatic activity under stress conditions (Pirasteh-Anosheh *et al*., 2015), consequently increasing yield characteristics. The application of SA also changes the hormonal balance in the plant and increases auxin and cytokinin levels in non-stress conditions. Furthermore, under stress conditions, this substance increases the amount of auxin and ABA while reducing cytokine reduction (Shakirova *et al*., 2003).

Research on the effect of SA treatment on various crop and yield components under drought stress has been reported to improve growth, vital processes in plants, antioxidant mechanisms, and defense systems (Ebrahimi & Jafari, 2012; Rafique *et al*., 2023; Sangwan *et al.*, 2022; Khan *et al.*, 2022; Tanveer *et al*., 2023).

Studies on the effect of SA on the mung bean plant (Ali & Mahmoud, 2013) and peanut (Karimian *et al*., 2015) revealed that SA level significantly increased grain yield vis-à-vis control. It also helps in maintaining the membrane under DS. The effect of DS and foliar SA spray-on black cumin has indicated that the maximum seed number per foliquol (66.33) was obtained in 90% field capacity and 10 µM SA treatment.

Leaf development is one of the most sensitive processes affected by water deficiency. Studies show that DS makes cells smaller and lessens the number of cells produced by meristems (Tardieu *et al*., 2000). Thus, it is natural that plants' metabolic processes diminish under water stress conditions, and in turn, growth indices reduce. Assessment of different amounts of soil moisture and SA levels on enzymatic activity and morphophysiological characteristics of alfalfa plant showed the highest number of leaves per plant in field capacity of 100%. Selection based on leaf area and plant biomass under DS conditions increased yield potential in maize (Pandey *et al*., 2017). The harvest index is one of the most critical physiological indices showing the percentage of photosynthetic transfer from the plant to its grains, and it varies during drought (Zecevic & Knezevic, 1997). In each environmental condition, seed yield per plant results from biomass and harvest index (Hegde *et al*., 2007). During the canola stem elongation stage, DS increased the harvest index as dehydration stress during stem elongation affects the production of dry matter straw more than grain yield (Wright *et al*., 1988).

Chlorophyll content in living plants is critical in maintaining photosynthetic capacity (Tommasino *et al*., 2018); however, it is affected by DS. The main factor that reduces photosynthesis during dehydration is reducing available CO_2, which limits the diffusion through the stomata and mesophyll (Posch *et al*., 2019). It is reported (Liang *et al*., 2020) that the application of 1.5 mM SA increased chlorophyll a, b, and total (Table 5). It has been proven that SA produces a comprehensive metabolic response in plants and affects their photosynthetic properties and water relations. It has also been reported that immersion of wheat seeds in SA under non-stress conditions increased pigments(Fariduddin *et al*., 2003).

Conclusion

The results showed that while DS poses negative impacts on foxtail millet yield and its components, SA plays a positive role in modulating the effects of DS and helps in promoting plant growth and yield parameters. Therefore, it can be stated that irrigation at 85% of the humidity of field capacity and 1.5 and 3 mM SA treatment can produce a good yield in Sistan's climate and similar weather conditions.

Informed consent

The authors declare that no patients were involved in this research.

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