

Minimizing the effect of low moisture stress in pearl millet (*Pennisetum glaucum* L.) by regulating growth, yield and antioxidant defense system via foliar applied silicon.

ABSTRACT

Most abundant element on earth crust is silicon (Si), easily available and mostly used in farming against low moisture stress in arid regions. Thus, a pot experiment was accomplished for identifying the beneficial effects of Si on the growth, productivity and activity of antioxidants in pearl millet. The millet plant was arranged in complete randomized design (CRD) under normal and drought stress condition and treated with four level of Si (0.0, 2.5, 5.0, and 7.5 mmol/L). The results revealed that foliar 5.0 mmol/L of foliar applied Si improved the growth traits (leaf fresh weight 18.99%, stem fresh weight 9.34%, root fresh weight 28.75%, root dry weight 33.11%, plant height 21.7%, and stem diameter 28.26%) and yield traits (no. of grains per spike 9.22%, and grain yield 15.89%). While it enhances the activity of antioxidants as (catalase 8.1%, peroxidase 36.84%, and ascorbate peroxidase 17.16%). In short, foliar application of Si is an effective strategy for improving all the growth and yield traits as well as some of the antioxidants in the presence of low moisture stress in pearl millet crop. In this manner, foliar applied 5.0 mmol/L of Si will be recommended as the best doze for controlling the negative impact on pearl millet crop. Hopefully, the findings of this research will be helpful for the future research against stress related challenges.

Keywords: Pearl millet, Silicon, Drought stress, Growth and yield traits, Antioxidants

1. INTRODUCTION

Tremendously depleting underground and aboveground water reserves as well as instant global environmental variations are the leading causes of abiotic stresses such as drought stress [1]. Same like other abiotic stresses, low moisture stress also effects the development of various species of crops. Because, in this situation intra-plant water level lower down to its optimum level [2]. Low moisture stress is one of the leading stresses affecting the productivity of various crops globally. The major changes in plant due to low moisture stress are physiological changes like loss of water contents and leaf water status as well as decline in turgor pressure, Morphological changes like reduction in growth and yield, and oxidative changes like variation in the activity of reactive oxygen species (ROS) [3]. Generally, pearl millet is cultivated earlier then the rainy season. Pearl millet crop has the ability to survive under low moisture stress during its early growth stages due to the extending and variable raining pattern. But it is sensitive to the drought stress during its reproductive stages[4]. That's way it is necessary to put attention towards the drought stress at the later stages of pearl millet [5]. Three different strategies are adopted by the plant to cope up from drought stress which are avoidance, escape, and tolerance. But these strategies are not enough to protect the plant from this harsh condition [6]. Agriculturists are utilizing various techniques as well as natural and synthetic resources to improve the growth and productivity of crops [7].

2nd most plentiful element in earth crust is silicon (Si) and comprises of almost 50 to 70 % of the soil. Si concentration varies from plant to plant ranging from 0.1 to 10 % of the total dry mass [8]. It is also known as the beneficiary mineral element for the plant, because it promotes the development of various plant

species under fluctuating climatic condition. Though, the uses of Si and its beneficial impact on plant are still confidential due to the variation among varieties, species, and climatic situation. Si is also known as extensively used drought stress lessening element in all type of plants. Si promote intra-plant physiological activities, through which plant recover its water balance [8]. When foliar application of Si was done in the presence of stress condition, Si improve the growth and productivity of the plant and create resistance to various types of abiotic stresses such as drought [2], salt [9], nutrient imbalance [10], freezing [11], metal toxicity [12], radiation damage [13], pests, and pathogens [14].

Pearl millet (*Pennisetum glaucum* L.) is known as C₄ dual purpose crop used for both forage and grain purpose and mostly grown in arid and semi-arid areas due to its superiority in tolerance to abiotic stresses [15]. It is also known as annual forage crop which is grown in summer and used for making hay, silage, green chop as well as used for grazing. It is mostly cultivated in the low rainfall areas of Asia and Africa [16]. In relation to the other cereals, pearl millet contains more zinc (Zn) and iron (Fe). That's way, it is widely used to feed the animals and humans [17]. Due to the high concentration of Fe, protein, antioxidants and other beneficial macro and micro molecules, pearl millet is also called as "Nutri-Cereals" [18]. Pearl millet also contribute in food security in the low rainfall areas but its contribution is not enough to optimize the growth and development in arid and semi-arid regions. That's way there is still a huge research gape to find the best method of adaptation of millet plant to water limited condition [19]. There is a need to find an osmotic regulating reagent, through which plant can normalize its water status under acute moisture stress [20].

In previous studies, various strategies were adopted to minimize the impact of low moisture stress on pearl millet plant. In this study, role of Si was checked in mitigating the drought stress due to its popularity in biotic and abiotic stress mitigation. The aims of the present study were (1) to check the surviving ability of pearl millet under drought stress, (2) Either Si is helpful in drought stress mitigation for pearl millet crop or not, and (3) Selecting the best doze of Si showing highest improvement in growth, yield, and oxidative changes in pearl millet.

2. MATERIALS AND METHODS

2.1 Research location and weather condition

A pot experiment was conducted at the greenhouse area of College of Agriculture, University of Layyah, Punjab, Pakistan (Latitude 31°01'18" N, Longitude 70°57'09" E) to examine the role of silicon in minimizing the effect of drought on pearl millet.

2.2 Experimental design, Arrangement of treatments and Drought Imposition

In this complete randomized design (CRD) two factorial design, 32 equal size and weight pots (70 cm tall and 30 cm wide) were selected and divided in to 4 replications as 8-pots per replication. Equal quantity of sandy loam soil was filled in each pot. Soil samples were collected and tested. The soil properties are clearly mentioned in (Fig. 1). Sowing was done during kharif season of 2020. Five seeds were sown in each pot but three plants were maintained after thinning. 90-45-0 kg/ha of NPK was applied through fertigation as per treatment. Each pot was watered equally. Two levels of irrigation were applied as (Normal irrigation and Skipped irrigation at spike initiation stage). As well as doses of silicon (0, 2.5, 5.0 and 7.5 mmol/L) was applied foliarly in the form of (Na₂SiO₃) purchased from Sigma-Aldrich Company.

2.3 Plant observations

2.4 Soil water content

After the completion of treatment period, the soil sample were collected from each pot and weighed instantly to get its wet weight. After that, same soil sample was dried at 90°C for 24 hours. Again, weighed the sample when soil was completely dry. These two values of wet and dry weights were then used to calculate soil water content (SWC). SWC was calculated as

$$SWC = \frac{[(Soil\ wet\ weight) - (Soil\ dry\ weight)]}{(Soil\ wet\ weight)} \times 100$$

2.5 Growth traits

At maturity, flag leaf of all the three plants were removed and their fresh weight was measured by using digital weighing balance. After that these leaves were used for analyzing antioxidants. One plant from each treatment was separated and the main parts such as (stem and root) were separated. Stem and root fresh weight and root dry weight was measured from these separated parts. Plant height and stem diameter was measured nondestructively by using measuring tape and digital Vernier caliper.

2.6 Yield traits

Grain per spike and grain yield of all the spikes from each treatment were measured separately and their average value was recorded. After that grain yield per spike was converted into kilogram per hectare.

2.7 Enzymatic antioxidant activity

2.7.1 Superoxide Dismutase (SOD) Activity

[21] described a method for the measurement of SOD activity. First of all, 1 ml of reaction mixture was prepared, which consist of 1 mM of ethylene-diamine tetra-acetic acid (EDTA), 0.05 molar phosphate buffer, 130 mM methionine, 0.75 mM nitro-blue tetrazolium (NBT) and 0.02 mM riboflavin. Prepared mixture was kept in fluorescent light for 7-minutes and at 560 nm, absorbance was recorded. Activity of SOD was measured by using Lambert Beer law:

$$A = \epsilon LC$$

Here A is nominating the absorbance rate, Extinction coefficient is mentioned by ϵ , L is showing the size of individual walls and C is the ratio of enzymes.

2.7.2 Catalases (CAT), Peroxidases (POD) and Ascorbate Peroxidase (APX) Activities

The method of [22] was utilized after doing a little bit change to determine the CAT activity. 0.5 mL of enzyme extraction was added to the reaction mixture (3 ml), Phosphate buffer (50 mM), pH was 7.0 and H_2O_2 @ 30 % w/v). CAT activity was analyzed when absorbance was minimized at 240 nm. POD activity was determined by utilizing the method of [23] as, Reaction mixture (3 ml) consist of Guaiacol (20 mM), Phosphate buffer (10 mM), and H_2O_2 (10 mM) was mixed with (0.5 mL) of enzyme extraction (which was warmed in water bath at 45°C for 5-minutes before stirring). Increment in absorbance was recorded @ 470 nm due to the creation of tetra-guaiacol [24]. APX activity was measured by following the work of [25].

2.8 Statistical Analysis

The whole data was assembled in Microsoft Excel. All the data was analyzed by using Fisher's two-way analysis of variance (ANOVA) technique. Least significant difference (LSD) test was used to estimate the mean values of each treatment[26] in Statistix 8.1. All the graphs were created in a software "Origin PRO 9.1" (Origin LabCorporation, Northampton, USA).

3. RESULTS

3.1 Soil water contents (SWCs) during drought stress and silicon application

SWCs measurements recorded at the time of treatments application are shown in (Fig. 2). Variation in SWCs measurements for whole Si treatments were not significantly different. Highest effect of 2.5 mmol/L of Si was found in 0 & 6-days after Si application. 5.0 & 7.5 mmol/L of Si application showed highest soil moisture contents in 3 & 9 days after Si application respectively.

3.2 Significance level of all the studied traits

Whole one way and two-way interactive study presented in (Table 1) showing that foliar application of silicon (Si) significantly enhances the growth (leaf fresh weight, stem fresh weight, root fresh weight, root dry weight, plant height, and stem diameter), yield (no. of grains per spike, and grain yield) and antioxidants concentration (catalase, peroxidase, ascorbate peroxidase, and superoxide dismutase) of pearl millet under both normal and drought stressed condition.

3.3 Individual study

It is found that individual effect of drought and Si-treatments significantly affected all the morphological traits and activity of antioxidants. Imposing drought stress to pearl millet plant decreased the growth traits as (leaf fresh weight 12.51%, stem fresh weight 11.24%, root fresh weight 21.63%, root dry weight 24.22%, plant height 9.78%, and stem diameter 16.14%) and yield traits as (no. of grains per spike 6.56%, and grain yield 5.31%). While it enhances the activity of antioxidants as (catalase 23.25%, peroxidase 30.33%, ascorbate peroxidase 12.19%, and superoxide dismutase 5.62%) as compared to the well water condition (Fig. 3).

After studying the individual effect of Si-treatments it is found that, foliar application of 5.0 mmol/L of Si significantly enhanced all the growth traits as (leaf fresh weight 18.99%, stem fresh weight 9.34%, root fresh weight 28.75%, root dry weight 33.11%, plant height 21.7%, and stem diameter 28.26%) and yield traits as (no. of grains per spike 9.22%, and grain yield 15.89%). While it enhances the activity of antioxidants as (catalase 8.1%, peroxidase 36.84%, ascorbate peroxidase 17.16%, and superoxide dismutase -12.8%) as compared to the controlled condition of Si (Fig. 4).

3.4 Interactive study

In morphological traits, maximum flag leaf and stem fresh weight was found by the application of 5.0 mmol/L Si followed by 7.5 mmol/L, 2.5 mmol/L, and 0 mmol/L under both well-watered and drought condition. Similarly, the same trend was also found in other growth traits (fresh and dry root weight, plant height, and stem diameter) and yield traits (no. of grains per spike and grain yield). Foliar application of 5.0 mmol/L of Si improve the flag leaf and stem fresh weight to 15.1% and 10.1% (Fig. 5), fresh and dry root weight 28.1% and 36.6% (Fig. 6), plant height and stem diameter 21.9% and 18.3% (Fig. 7) as well as no. of grains per spike and grain yield 9.8% and 12.9% (Figure 8) respectively under well-watered condition. On the other hand, application of Si at the rate of 5.0 mmol/L under drought stress condition enhanced all the morphological traits as flag leaf fresh weight (23.4 %), stem fresh weight (8.5 %), fresh root weight (29.5 %), dry root weight (28.4 %), plant height (21.4 %), stem diameter (38.8 %), no. of grains per spike (8.6 %) and grain yield (19.0 %) (Fig.5, 6, 7, and 8).

After analyzing the concentration of antioxidants (catalase, peroxidase, ascorbate peroxidase, and superoxide dismutase) under well-water as well as drought stress condition, it was found that drought stress condition significantly enhance the concentration of antioxidants in leaves of pearl millet as compared to the well water condition. On the other side, application of Si increases the concentration of (catalase, peroxidase, and ascorbate peroxidase) and decrease the concentration of (superoxide dismutase). An increment of (16.73 %) in catalase, (38.97 %) in peroxidase and (19.15 %) in ascorbate peroxidase was observed by the application of (5.0 mmol/L of Si) as compared to the control (0 mmol/L of Si) under drought stress condition. While, this concentration decreases the concentration of (superoxide dismutase) to (12.33 %) as compared to the control condition (Fig. 9 and 10).

3.5 Correlation analysis

Across drought stress and Si-treatments, there was a strong positive correlation ($p < 0.01$, $r = 0.75$) among leaf fresh weight, stem fresh weight, root fresh weight, root dry weight, plant height, stem diameter, no. of grains per spike, and grain yield. While growth and yield parameters were showing negative correlation with antioxidants (CAT, SOD, APX, and POD). It means increment in the activity of antioxidants due to drought stress may decrease the growth and yield of pearl millet (Fig. 11).

4. DISCUSSION

Pearl millet is ranking sixth in the list of most valuable cereal crops of arid and semi-arid areas of Asia [15]. Drought stress act as a limiting factor and badly effect the production via disturbing the physiological and biochemical functioning of plant [27]. Exogenous application of Si act as a drought mitigating agent [28]; by improving the physiological functioning of pearl millet [2].

Different type of seasonal variations caused by drought related conditions greatly effects the fresh and dry biomass (FLFW, SFW, FRW, and DRW), plant height, and diameter of pearl millet (Fig. 3), [29]. A significant reduction in plant length and stem diameter was observed due to the low moisture stress at reproductive stage of pearl millet [2]. Same results were also found by the study of [30] as pearl millet can tolerate a specific range of drought stress by doing some morphological and physiological changes such as opening and closing of stomata, photosynthetic activity, changing the time of phenological stage, reducing the leaf and root properties, and activating the antioxidative defense mechanism. Under drought stress condition, low water use efficiency affect the plant height, stem diameter, shoot and root traits, crop physiology, and fruiting of pearl millet [29]. But according to other scientists, pearl millet is a stress tolerant and climate friendly crop which can be grown in any type of environmental condition [31,32].

Root uptake different types of macro and micro nutrients from the soil and transfer it to the leaves and leaf creates different kind of assimilates and transfer it to the grains. The linkage among the development of grains and assimilates transformation largely depends on the availability of moisture [33]. A huge loss in yield attributes was found when plants were exposed to low moisture stress at reproductive stage. Flowering stage is a critical growth stage where impact of drought stress was too high [34]. A huge yield gap was observed when crop was exposed to low moisture stress at flowering initiation stage [35]. The raining period start and end during the vegetative stage, that's way drought stress mostly occurs at reproductive stage. Actually, terminal drought stress is well known for its devastating effect on the productivity of pearl millet crop [36]. The current experimental study also showed the same results as previously described that; pearl millet requires adequate quantity of soil moisture to compete with terminal drought stress. Otherwise, pearl millet adopts various moisture saving strategies as the accumulation of reactive oxygen species (ROS) [37,38]. A major change in plant due to drought stress is the increasing level of reactive oxygen species (ROS), which is responsible for the oxidative damage in plant such as increasing level of antioxidants (CAT, SOD, POD, and APX) [39]. Drought stress allow huge accumulation of ROS which result in cellular variation in plant [40].

In short, pearl millet shows different type of visual and non-visual symptoms: (a) Growth and yield traits such as plant height, stem diameter, grain yield, and yield related traits; (b) Morphological traits such as flag leaf, shoot and root related traits; (c) Physiological traits (d) Biochemical related traits such as antioxidants level in plant and the presence of ROS under drought stress condition [41].

Exogenous application of plant beneficial material is required to cope the damage caused by low moisture stress in soil. In this study Si was applied exogenously as an essential nutrient for improving the growth and productivity and to create tolerance in pearl millet crop (Figure 4). Exogenous application of Si enhances the growth, physiological, and yield traits of millet crop [42,28]. Foliar application of Si also prompts oxidative changes by minimizing the oxidative stress and finally assist the plant under low moisture stress [43]. According to [2], all the growth and yield traits of millet crop such as (plant height, stem diameter, yield, and yield related traits) were decreased under drought stress. 13-19% decrease in thousand grain weight and total grain yield was observed in drought stressed millet crop as compared to the normal crop. Some physiological changes in plant due to drought stress are the main cause of this loss [44]. Si application is an effective strategy which increase the yield and yield related attributes of millet crop (Figure 8). Foliar applied Si improve 10 percent of grain yield in comparison to the control condition. Si application improve water, light, and nutrient use efficiency of crop and enhance the yield [45]. It also improves the tillering ability, spike length as well as helpful in maintaining the leaves properties [46].

Foliar applied Si significantly enhance the activity of antioxidants (POD, CAT, and APX) under abiotic stress condition in millet crop (Figure 9 and 10). [47] confirm that activity of POD and CAT in the leaves and root of millet crop increased due to the application of Si under salinity and drought condition. The same results were also gained by the study of [48] in cowpea crop under drought stress condition. Drought stress lower down the transpiration rate by minimizing the stomatal conductance, which directly

affect the photosynthesis process and enhance the level of ROS and antioxidants [49]. Application of Si improve the activity of CAT, POD, and APX, which is the first line of defense mechanism helpful against stress condition. [50,51,52] confirmed the results in their studies on wheat, bean, and alfalfa crops respectively.

5. CONCLUSION

The result revealed that foliar applied silicon significantly boosts up the morphological and biochemical attributes of pearl millet under drought stress condition. It improves all the growth traits (flag leaf fresh weight, stem fresh weight, fresh root weight, dry root weight, plant height, and stem diameter), yield traits (grain per spike and grain yield), and antioxidants (catalase, peroxidase, and ascorbate peroxidase) in the presence of low moisture stress. Among the studied doses of silicon, 5.0 mmol/L of foliar applied Si is selected as the best preforming dose not only under normal condition but also under drought stress condition. Therefore, findings suggest foliar spray of silicon as an effective strategy against drought stress. Though, further experimental work is still required to identify the uses of Si against salinity, heat, and heavy metal stresses for other cereal and oil seed crops.

DATA AVAILABILITY STATEMENT

All obtained data is enclosed with this manuscript.

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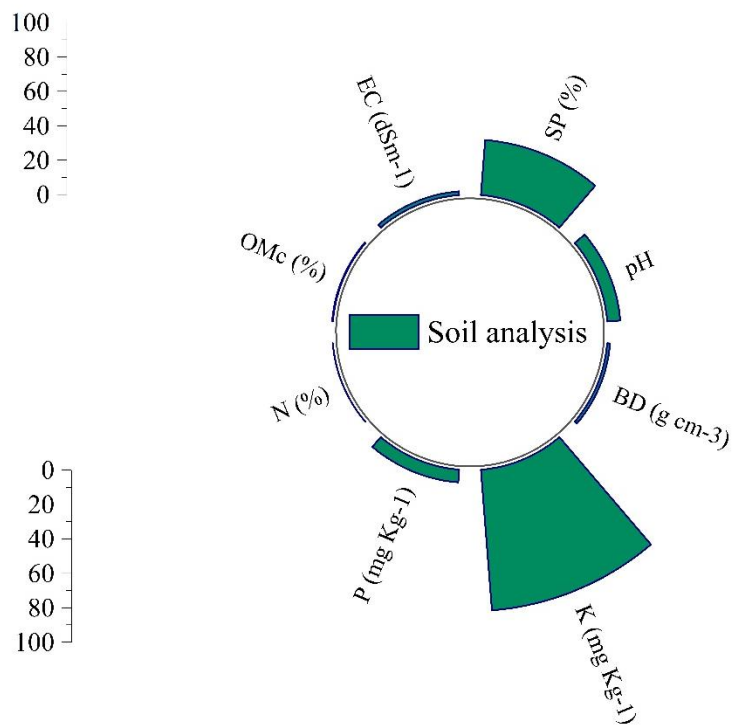
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Table 1. Significance levels of all the studied traits under drought stress condition and CaCl₂ application.

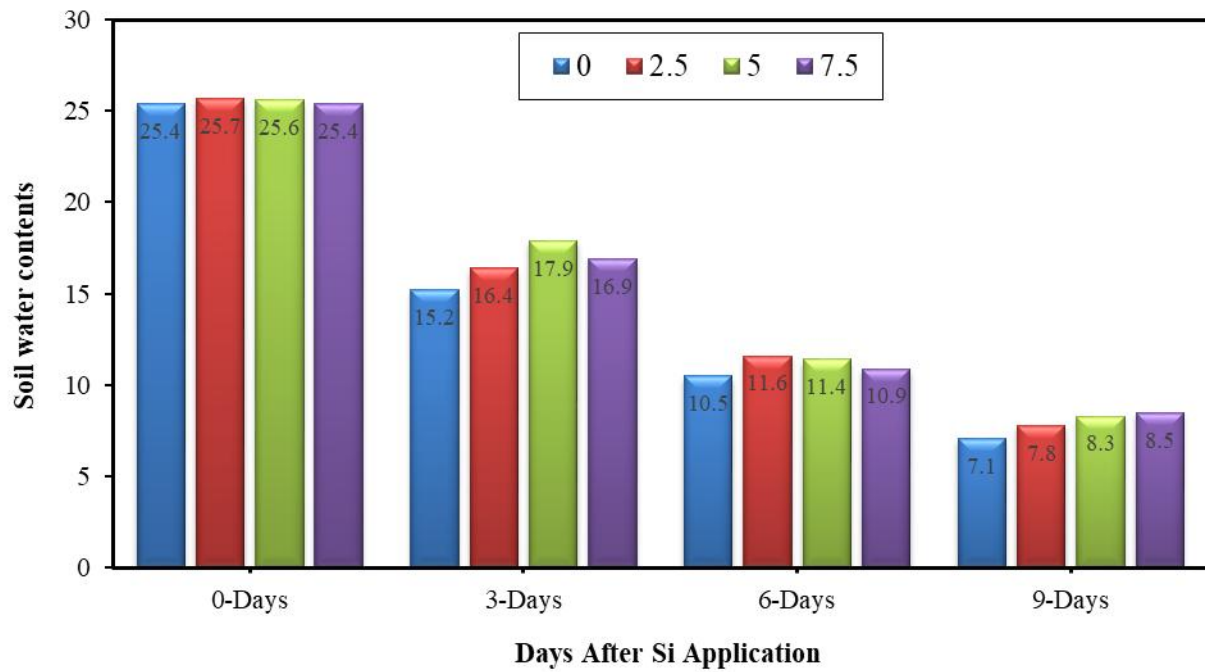
No.	Studied Parameters	Treatments applied		
		Drought levels (D)	CaCl ₂ levels (T)	D × T
1	Leaf fresh weight	**	**	*
2	Stem fresh weight	**	**	*
3	Root fresh weight	**	**	*
4	Root dry weight	**	**	*
5	Plant height	**	**	**
6	Stem diameter	**	**	**
7	No. of grains per spike	**	**	**
8	Grain yield	**	**	**
9	Catalase	**	**	**
10	Peroxidase	**	**	**
11	Ascorbate Peroxidase	**	**	**
12	Superoxide dismutase	**	**	*

Fig. 1. Mean of soil analysis report of randomly selected pots.



SP, saturation percentage; BD, bulk density; K, potassium; P, phosphorus; N, nitrogen; OMc, organic matter contents; EC, electrical conductivity

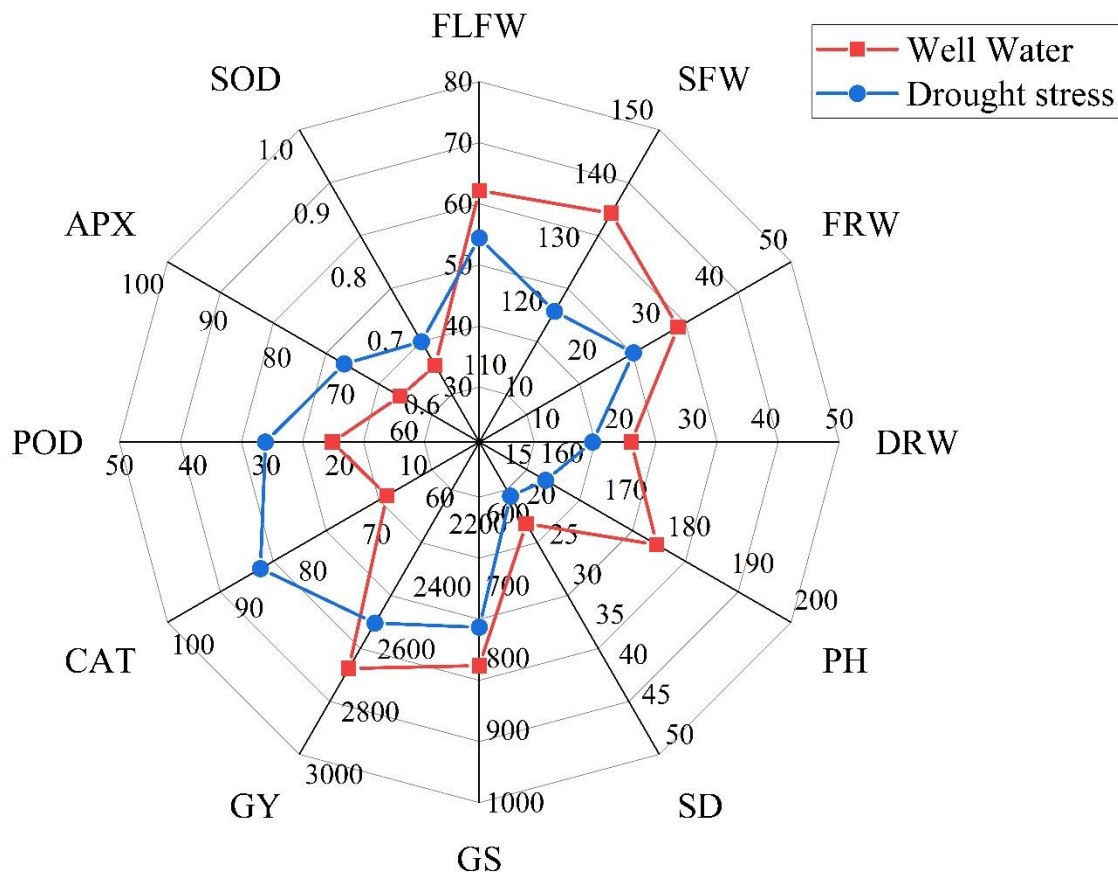
Fig. 2. Effect of silicon application on soil water contents during drought period.



0, 0 mmol/L of Si; 2.5, 2.5 mmol/L of Si; 5, 5.0 mmol/L of Si; 7.5, 7.5 mmol/L of Si

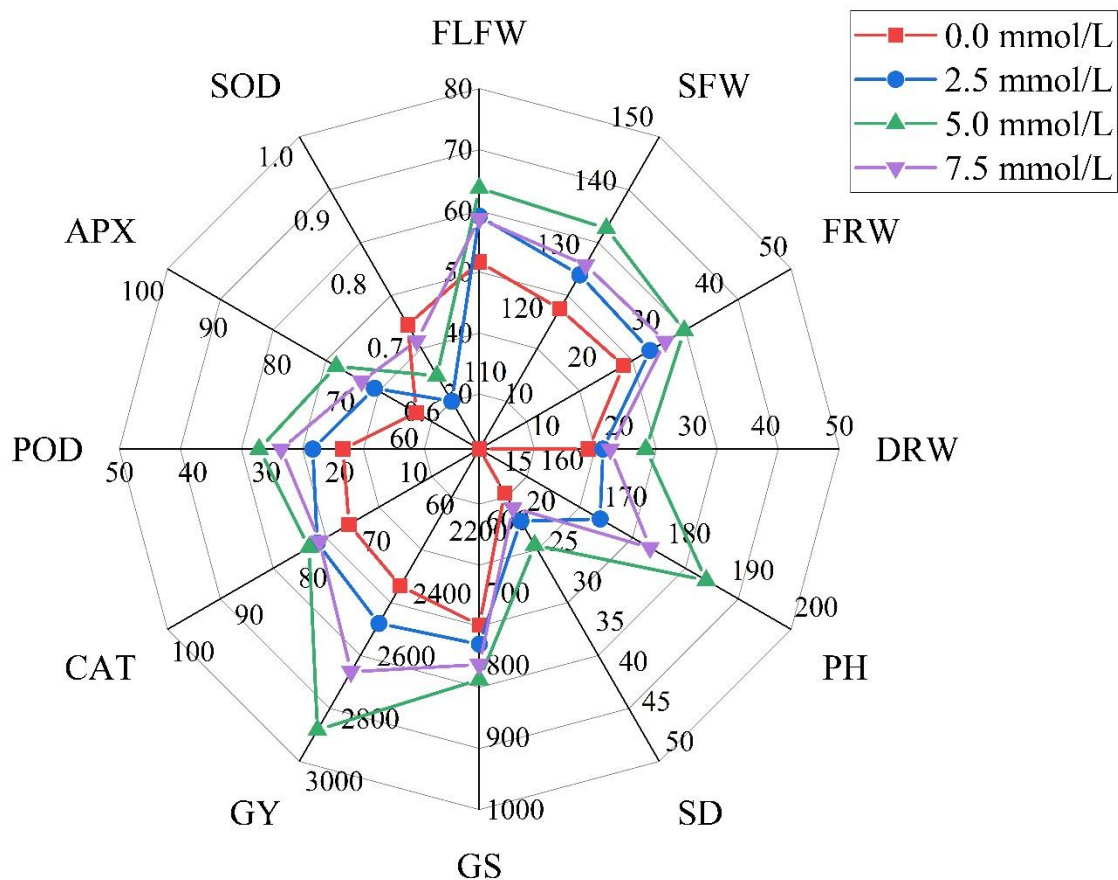
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Fig. 3. Radar graph showing the effect of different levels of irrigation regime on growth, yield, and antioxidant traits of pearl millet.



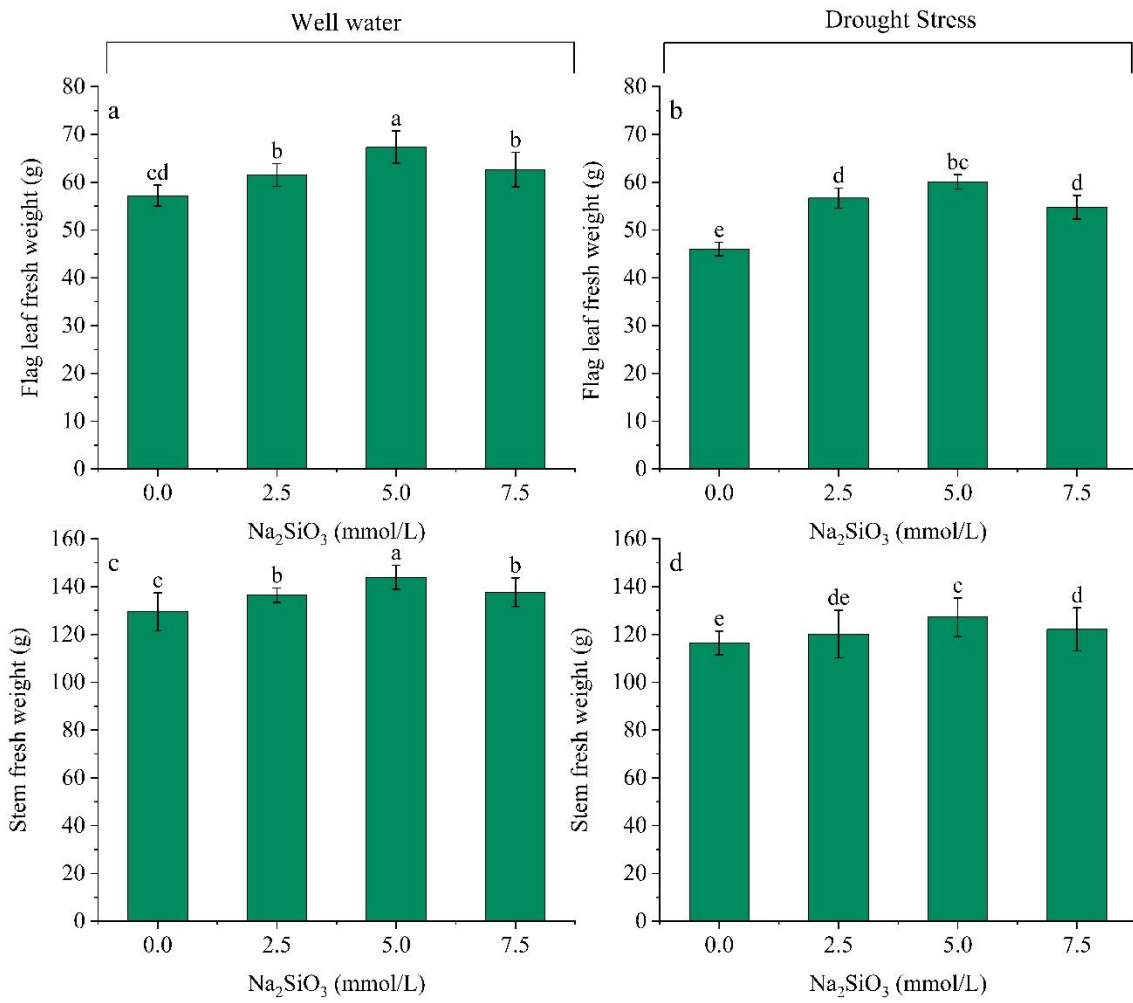
FLFW, flag leaf fresh weight; SFW, stem fresh weight; FRW, fresh root weight; DRW, dry root weight; PH, plant height; SD, stem diameter; GS, grain per spike; GY, grain yield; CAT, catalase; POD, peroxidase; APX, ascorbate peroxidase; SOD, superoxide dismutase.

Fig. 4. Radar graph showing the effect of different levels of foliar applied silicon on growth, yield, and antioxidant traits of pearl millet.



FLFW, flag leaf fresh weight; SFW, stem fresh weight; FRW, fresh root weight; DRW, dry root weight; PH, plant height; SD, stem diameter; GS, grain per spike; GY, grain yield; CAT, catalase; POD, peroxidase; APX, ascorbate peroxidase; SOD, superoxide dismutase.

Fig. 5.Effect of foliar applied silicon on the flag leaf fresh weight and stem fresh weight of pearl millet under drought stress.



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Fig. 6. Effect of foliar applied silicon on the root fresh weight and root dry weight of pearl millet under drought stress.

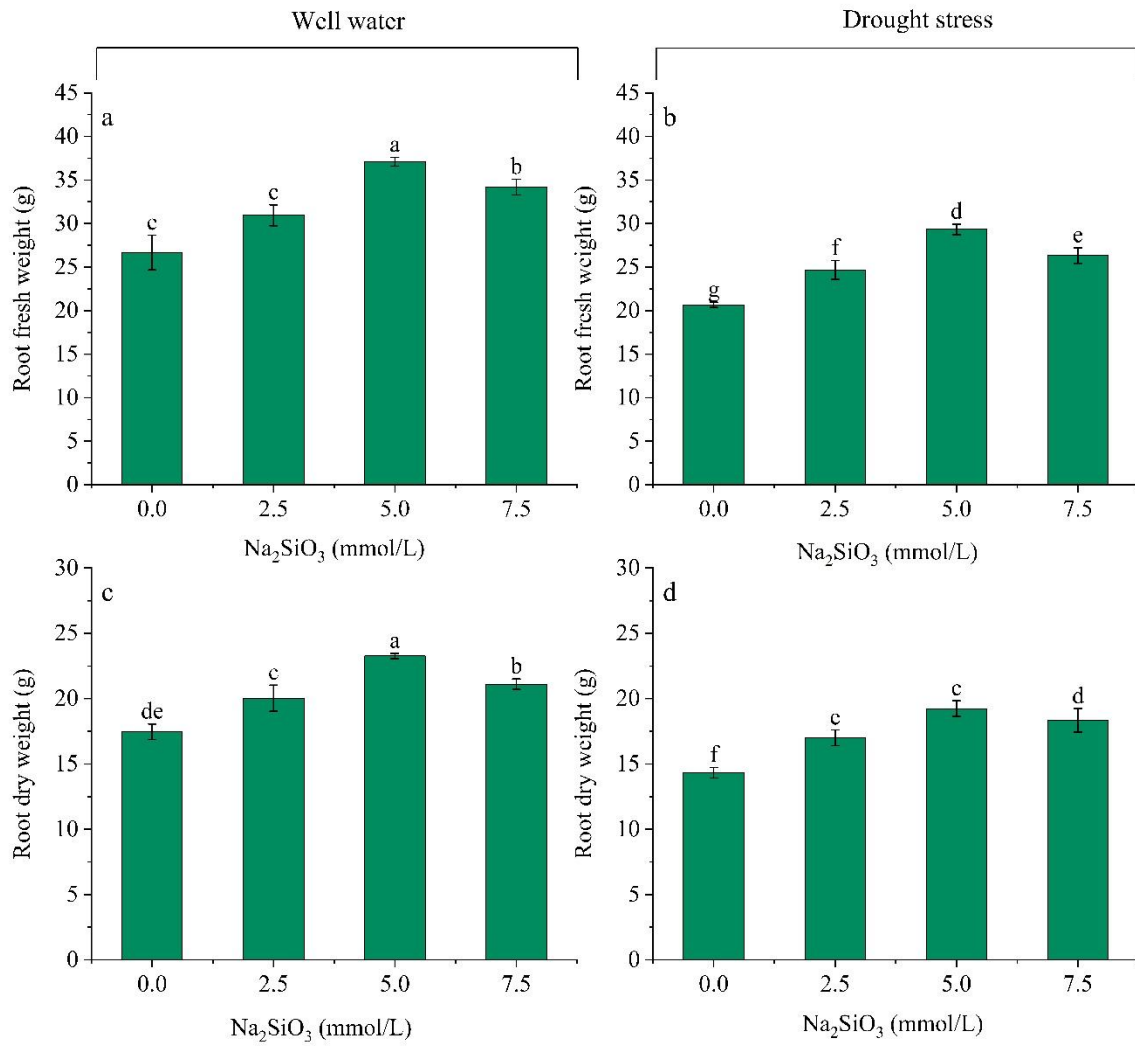


Fig. 7.Effect of foliar applied silicon on the plant height and stem diameter of pearl millet under drought stress.

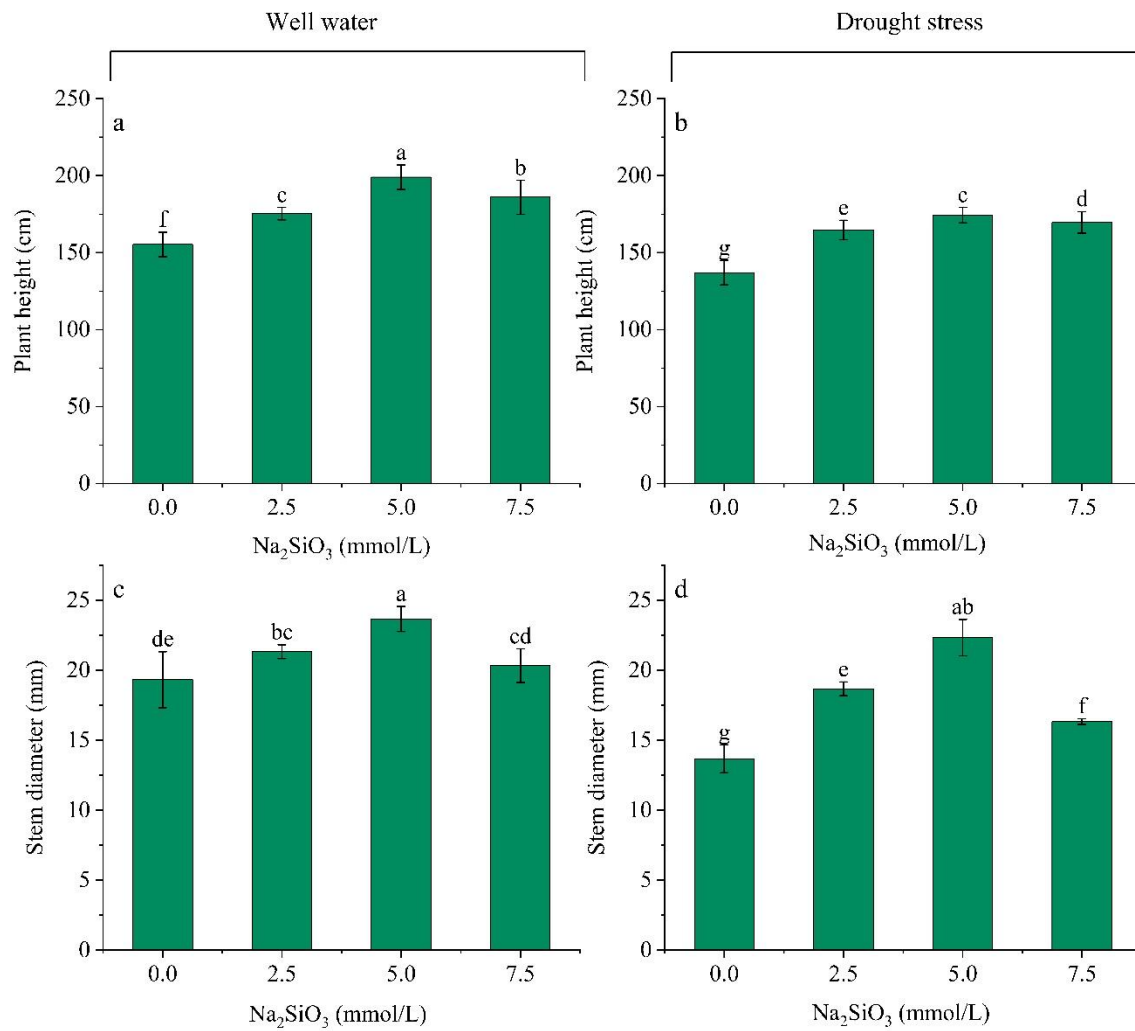
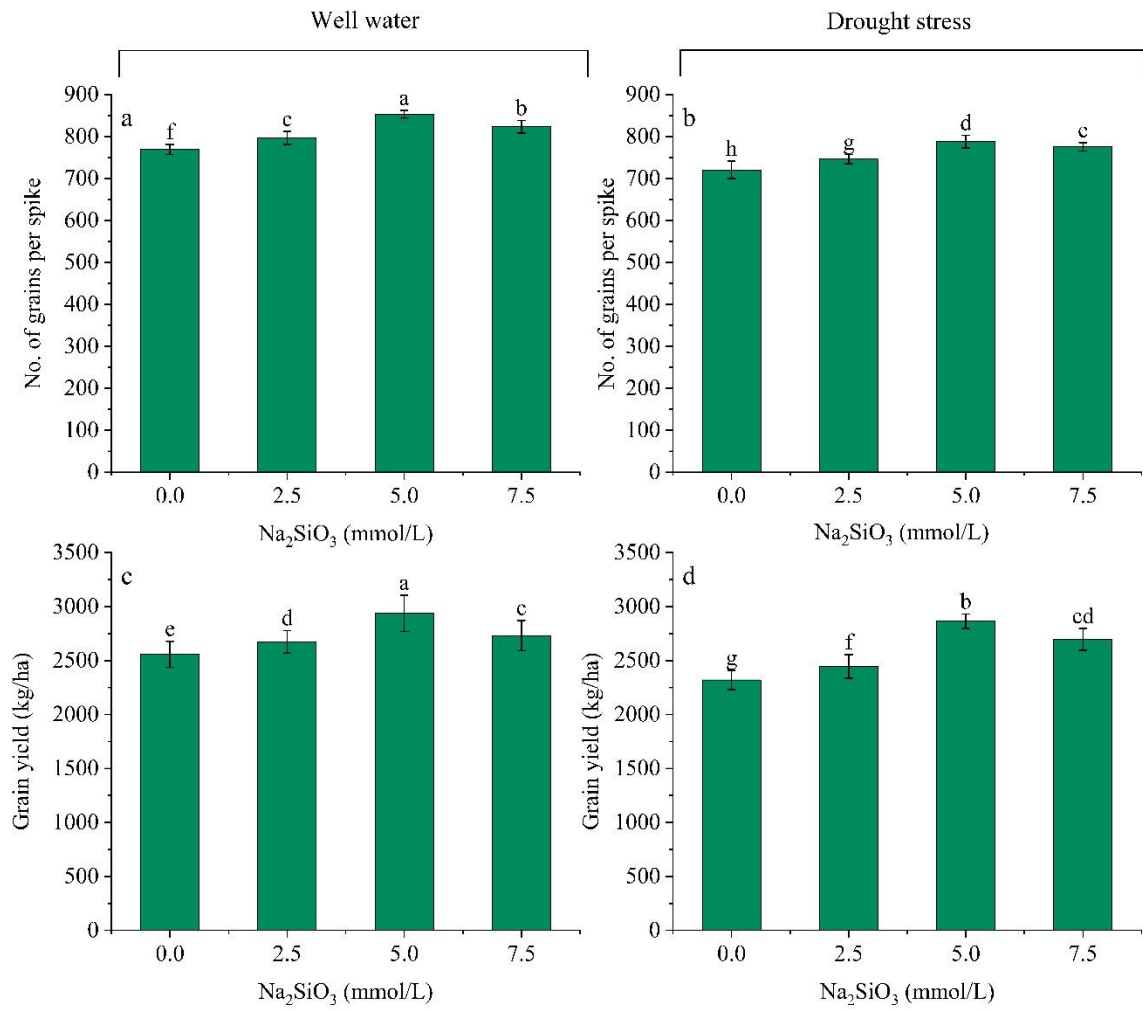


Fig. 8.Effect of foliar applied silicon on the no. of grains per spike and grain yield of pearl millet under drought stress.



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Fig. 9.Effect of foliar applied silicon on the catalase and peroxidase of pearl millet under drought stress.

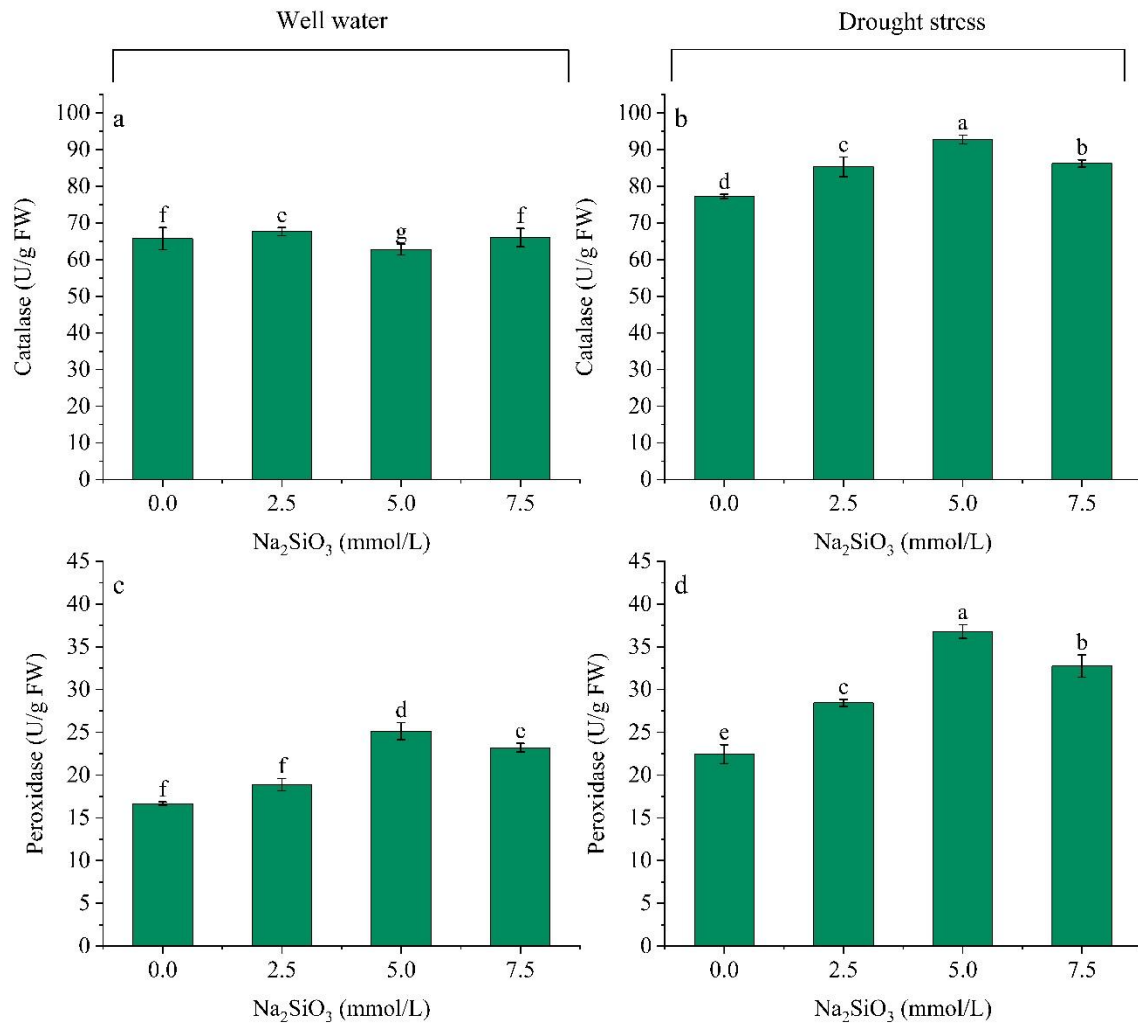


Fig. 10.Effect of foliar applied silicon on the ascorbate peroxidase and superoxide dismutase of pearl millet under drought stress.

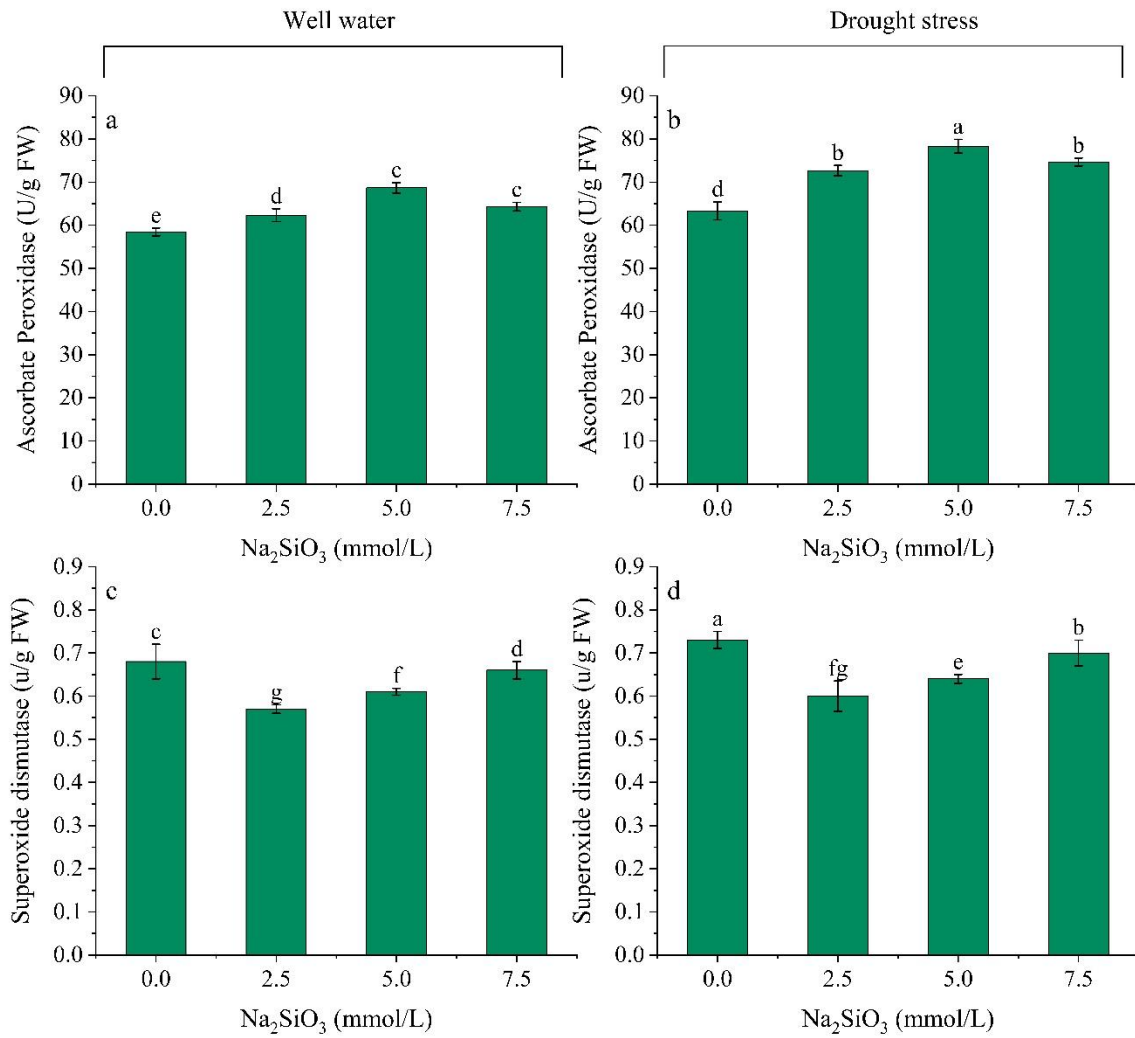


Fig. 11. Correlation between growth, yield and antioxidant traits under two level of drought stress (well water and drought stress) and four levels of Si-treatment (0.0, 2.5, 5.0, and 7.5 mmol/L).

	FLFW	SFW	FRW	DRW	PH	SD	GS	GY	CAT	POD	APX	SOD
FLFW	↑ 1											
SFW	↑ 0.91	↑ 1										
FRW	↑ 0.9527	↑ 0.9601	↑ 1									
DRW	↑ 0.8976	↑ 0.9339	↑ 0.9584	↑ 1								
PH	↑ 0.9443	↑ 0.8429	↑ 0.9504	↑ 0.885	↑ 1							
SD	↑ 0.9453	↑ 0.8288	↑ 0.8575	↑ 0.8519	↑ 0.832	↑ 1						
GS	↑ 0.9316	↑ 0.9443	↑ 0.992	↑ 0.9499	↑ 0.9496	↑ 0.8233	↑ 1					
GY	↑ 0.8471	↑ 0.7555	↑ 0.8654	↑ 0.8307	↑ 0.8854	↑ 0.8273	↑ 0.8939	↑ 1				
CAT	↓ -0.423	↓ -0.713	↓ -0.528	↓ -0.541	↓ -0.303	↓ -0.295	↓ -0.51	→ -0.13	↑ 1			
POD	→ 0.0087	↓ -0.298	→ -0.039	→ -0.05	→ 0.1993	→ 0.0666	→ -0.002	→ 0.356	↑ 0.8418	↑ 1		
APX	→ 0.0888	↓ -0.248	→ 0.0067	→ -0.003	→ 0.2677	→ 0.1429	→ 0.0365	→ 0.3748	↑ 0.8166	↑ 0.9834	↑ 1	
SOD	↓ -0.71	↓ -0.547	↓ -0.542	↓ -0.506	↓ -0.603	↓ -0.748	↓ -0.47	↓ -0.401	→ 0.1755	→ 0.0443	→ -0.106	↑ 1

FLFW, flag leaf fresh weight; SFW, stem fresh weight; FRW, fresh root weight; DRW, dry root weight; PH, plant height; SD, stem diameter; GS, grain per spike; GY, grain yield; CAT, catalase; POD, peroxidase; APX, ascorbate peroxidase; SOD, superoxide dismutase.