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VARIATIONS OF YIELD, BIOCHEMICAL AND ANTIOXIDATIVE RESPONSES IN SESAME WITH SILICON AND CYTOKININ TREATMENTS UNDER DROUGHT STRESS

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ABSTRACT

Drought is one of the major limiting factors for crops that severely reduce plant growth and productivity. The application of cytokinin (Ck) and silicon (Si) fertilizers can help increase tolerance to drought stress in sesame plants. The present study aimed to evaluate the effects of Ck and Si fertilizers on seed yield, malondialdehyde (MDA) content, proline content, and antioxidant enzyme activities in sesame plants under drought-stress conditions. The experiment was conducted as a split plot-factorial in a randomized complete block design with three replications at Firuzkandeh Agricultural Research Station during two crop years of 2020 and 2021. The main plot was three drought stress levels: control, moderate drought stress (MDS), and severe drought stress (SDS), whereas the subplots were three Si application levels: control or non-use of Si, calcium silicate and nano-Si, and two Ck application levels: control or non-use of Ck, Ck application. The results indicated that the sesame seed yield was reduced by 9.3% under MDS and by 32.7% under SDS when compared with control conditions. The highest MDA content and proline accumulation were observed when the plants were subjected to SDS, whereas the higher activity of antioxidant enzymes occurred under MDS. Higher activity of antioxidant enzymes and reduction of MDA content was observed in the plants treated by combined application of Si and Ck under MDS. However, the higher seed yield, greater proline content, and higher antioxidant enzyme activities were obtained from plants treated by nano-Si than calcium silicate. Overall, the results of the present study revealed that the foliar application of nano-Si + Ck can be a promising option for mitigating the negative impacts of drought stress and improving sesame seed yield.

Key words: antioxidant enzymes, malondialdehyde, nano-Si, proline, seed yield

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops, which is usually cultivated in arid and semi-arid regions of the world [Eskandari et al. 2009]. Drought stress is one of the most destructive abiotic stresses that lead to physiological and biochemical changes in the plant and subsequently reduce plant productivity [Yousefzadeh Najafabadi and Ehsanzadeh 2019]. Drought is one of the most critical environmental stresses that severely limit plant growth and production [Khalvandi et al. 2021]. Gholipur Noveyri et al. [2022] demonstrated that the decrease in moisture level caused a reduction in the yield of sesame seeds by affecting the physiological characteristics of the plant. Yousefzadeh Najafabadi and Ehsanzadeh [2019] found that the drought stress caused a decrease in the growth, yield components, and

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yield of sesame plants by reducing the concentration of photosynthetic pigments. In recent years, various approaches have been used to reduce the adverse effects of drought stress on crops. Cytokinin (Ck), as a phytohormone, plays an essential role in regulating the growth and photosynthetic system stability of plants under drought stress and modulating many physiological activities induced by drought stress [Werner et al. 2010]. Ck induces plant drought coping mechanisms by increasing relative leaf water content [Zaheer et al. 2019], enhancing photosynthetic pigments concentration [Opabode and Owojori 2018], and increasing antioxidant enzyme activities [Sepehri and Rouhi 2017]. It has been reported that Ck helps plant tolerance against drought stress by increasing the content of the photosynthetic pigment and reducing electrolyte leakage [Salek Mearaji et al. 2020].

Silicon (Si) is one of the beneficial elements for plants that can increase plant resistance against abiotic stresses like drought. The beneficial effect of Si in plants is related to its protective role in improving plant growth and yield under stress conditions [Bukhari et al. 2021]. Si increases plant resistance against drought stress by increasing the chlorophyll concentration and improving the photosynthesis rate [Cao et al. 2015]. It is documented that the Si application enhances the resistance of plants under drought stress by increasing the antioxidant enzyme activities [Zhu and Gong 2014]. The vital role of Si in reducing the adverse impacts of drought stress in plants such as canola [Bukhari et al. 2021], rice [Mauad et al. 2016], wheat [Maghsoudi et al. 2016, Ahmad et al. 2020], sorghum [Yin et al. 2014] and stevia [Askarnejad et al. 2019] have been reported.

Due to the lack of sufficient information on the physiological and biochemical responses of sesame plants to Si and Ck applications under drought stress, the present study aimed to evaluate the impacts of Si and Ck on the yield, malondialdehyde content, proline content, and antioxidant enzymes activities in sesame under drought stress conditions.

MATERIAL AND METHOD

Plant material and treatments

This experiment was performed at the Firuzkandeh Agricultural Research Station in Sari, Mazandaran Province, Iran (36°38’N, 53°41’E; 12.4 m above mean sea level) during the two crop years of 2020 and 2021. Soil physical and chemical properties are shown in Table 1.

This experiment was carried out as a split plot-factorial in a randomized complete block design with three replications. In this research, the main plot was three drought stress levels: control or without stress, moderate drought stress (MDS) and severe drought stress (SDS), whereas the subplots were three Si application levels: control or non-use of Si, calcium silicate (produced by TETACO company, Iran) and nano-Si (produced by S.D.P. company, France), and two Ck application levels: control or non-use of Ck and Ck (produced by SIGMA-ALDRICH company, Germany) application. The MDS and SDS were applied by irrigation after 100 and 200 mm evaporation levels from a class A evaporation pan, respectively.

Sesame seeds (Naz Tak-Shakhe genotype produced by an oil seeds company, Iran) were used as plant material in this experiment. The seedbed preparation was performed by plowing, harrowing, and disking the soil in the field. Then, the experimental field was divided into three equal replications, and each replicate had 18 plots. Each experimental plot consisted of 6 planting rows with row spacing of 50 cm and a length of 5 m. The distance between plants on the row was 4 cm. All the experimental plots received phosphorus at the rate of 80 kg P_2O_5 ha^{-1} as triple superphosphate before the sowing based on soil analysis results. Nitrogen fertilizer (ammonium sulfate 150 kg ha^{-1}) was applied in all plots at three splits (½ as basal fertilizer, ¼ as topdressing at the three-leaf stage, and ¼ as topdressing at five-leaf stage) based on soil analysis results. The calcium silicate was applied as basal at the rate of 400 kg ha^{-1}. The nano-Si and Ck at a concentration of 60 and 80 mg L^{-1}, respectively, were sprayed on sesame plants at the early flowering stage and continued once every two weeks until two weeks before harvest.

Traits measurement

Seed yield. At the maturity stage, plant samples in the central four rows of each plot were hand-harvested to determine sesame seed yield.

Malondialdehyde. Malondialdehyde (MDA) was determined using a procedure described by Heath and Packer [1968]. Fresh leaf tissue (0.50 g) was homog-

Table 1. Physical and chemical properties of the soil

Available K (ppm)	Available P (ppm)	Organic matter (%)	Organic carbon (%)	pH	EC (ds.m^{-1})	Clay (%)	Silt (%)	Sand (%)	Soil texture
233	73.6	2.46	1.43	7.09	0.84	22	34	44	loam

enized with 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 3,000 g for 5 min. A 1 ml aliquot of the supernatant was added to a test tube with 1 ml of thiobarbituric acid (TBA at 0.5% in TCA at 20%). The mixture was heated at 95°C for 30 min and then quickly cooled on an ice bath and centrifuged at 10,000 g for 5 min. The MDA content was calculated from the absorbance at 532 nm (correction was made by subtracting the absorbance at 600 nm for non-specific turbidity) by using an extinction coefficient of 155 mM^{-1} cm^{-1}. The MDA content was measured as µmol g^{-1} FW.

Proline content. Proline content was determined according to the method described by Bates et al. [1973]. Briefly, approximately 0.50 g of a fresh leaf was homogenized in 10 ml of 3% (w/v) sulfosalicylic acid, and then the homogenate was centrifuged at 10,000 g for 10 min. Then, 2 ml of the supernatant was mixed with 2 ml of acid ninhydrin solution and 2 ml of glacial acetic acid. The tubes were heated in a boiling water bath for 1 h. The reaction was then stopped by placing the tubes on ice. The mixture was extracted with 4 ml of toluene, and the absorbance of the upper layer was read at 520 nm by a spectrophotometer. The proline content in the test samples was calculated using a standard curve and expressed as mg g^{-1} FW.

Antioxidant enzyme activity. To extract antioxidant enzymes, the sesame fresh leaves (0.50 g) were homogenized in ice-cold solution with 50 mM potassium phosphate (pH 7.0) and 10 g polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000 g for 10 min at 4°C. The supernatant was used to determine the activities of catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX). CAT (EC 1.11.1.6) activity was assayed according to the method described by Aebi [1984]. The decomposition of H2O2 was monitored by the decrease in absorbance at 240 nm by spectrophotometer (Extinction Coefficient = 0.039 mM^{-1} cm^{-1}) and expressed as unit mg-1 protein. APX (EC 1.11.1.11) activity was determined according to the method described by Nakano and Asada [1981], based on the decrease in absorbance at 290 nm as ascorbate (ASC) was oxidized. APX activity was calculated by using the extinction coefficient of 0.039 mM^{-1} cm^{-1} and expressed as unit mg^{-1} protein. GPX (EC 1.11.1.9) activity was assayed by the method of Velikova et al. [2000] by measuring the absorbance increase at 470 nm (Extinction Coefficient = 26.6 mM^{-1} cm^{-1}), and the activity was expressed as unit mg^{-1} protein.

Statistical analysis

Statistical analysis was performed using SAS (ver 9.2) software. A combined analysis of variance was conducted as a split-plot factor in a randomized complete block design (RCBD) with three replications. Means were compared by the least significant difference (LSD) test at a 5% probability level.

RESULTS AND DISCUSSION

Seed yield. The results of combined analysis of variance showed that the main effects of year, drought stress (DS), silicon (Si), and cytokinin (Ck) were significant ($P \leq 0.01$) on seed yield, malondialdehyde (MDA) and proline content in sesame. Also, the MDA content was significantly ($P \leq 0.05$) affected by the three-way interaction between Ds × Si × Ck (Tab. 2).

In the present study, the seed yield in the second year of the experiment was 11.7% higher than in the first year. Our finding indicated that seed yield decreased significantly when drought stress increased. The highest seed yield (1750.6 kg ha^{-1}) was obtained under control conditions, while the yield was reduced by 9.3% and 32.7% under MDS and SDS, respectively. The results suggested that plants under SDS had significantly lower grain yield than those under MDS (Tab. 3). The decline in grain yield under water stress conditions compared with regular irrigation can be attributed to the reduction of photosynthetic materials in the plant under stress [Mohsennia and Jalilian 2012].

Table 2. Combined analysis of variance for drought stress, silicon, and cytokinin, as well as their interactions on seed yield, malondialdehyde, and proline content in sesame

Source of variation	df	Seed yield	Malondialdehyde	Proline
Year (Y)	1	949218.75**	1751.27**	0.085**
Replication (R)	4	9737.96	0.77	0.020
Drought stress (DS)	2	3126611.34**	673.60**	0.041**
Y × DS	2	15677.08^{ns}	106.59**	0.021**
Error	8	56899.07	6.41	0.006
Silicon (Si)	2	846694.67**	1229.08**	0.061**
Y × Si	2	1713.19^{ns}	3.85^{ns}	0.016**
DS × Si	4	13087.73^{ns}	140.48**	0.0004^{ns}
Y × DS × Si	4	6198.61^{ns}	10.96^{ns}	0.0052^{ns}
Cytokinin (Ck)	1	286752.08**	628.04**	0.064**
Y × Ck	1	555.78^{ns}	28.60^{ns}	0.009*
DS × Ck	2	6275.69^{ns}	31.17^{ns}	0.001^{ns}
Y × DS × Ck	2	1557.17^{ns}	21.57^{ns}	0.0004^{ns}
Si × Ck	2	17067.36^{ns}	354.56**	0.002^{ns}
Y × Si × Ck	2	2857.17^{ns}	98.31**	0.001^{ns}
DS × Si × Ck	4	4645.13^{ns}	46.97*	0.001^{ns}
Y × DS × Si × Ck	4	4823.14^{ns}	24.55^{ns}	0.0008^{ns}
Error	60	17725.65	17.91	0.002
CV (%)	–	8.84	12.75	11.93
SE	–	30.77	0.90	0.008

^{ns}, *, and ** are non-significant and significant at the 5 and 1% probability levels, respectively

Table 3. Mean comparison of main effects of year, drought stress, silicon, and cytokinin on seed yield and proline content in sesame

Experimental treatments		Seed yield (kg ha^{-1})	Proline (mg g^{-1} FW)
Year	2020	1411.8b	0.37b
	2021	1599.3a	0.42a
Drought stress	Control	1750.6a	0.37b
	MDS	1587.5b	0.38b
	SDS	1178.6c	0.43a
Silicon	Control	1342.3c	0.35c
	Calcium silicate	1527.7b	0.39b
	Nano-Si	1646.6a	0.43a
Cytokinin	Control	1454.0b	0.37b
	Cytokinin	1557.1a	0.42a

The means with the same letter(s) do not differ statistically by LSD test at P ≤ 0.05
MDS – moderate drought stress, SDS – severe drought stress

The findings in our study are in good agreement with previous studies in sesame [Dossa et al. 2017] that the seed yield decreased when the crop was subjected to drought stress.

We found that the application of Si in both calcium silicate and nano-Si forms significantly improved the seed yield compared with the control. Application of calcium silicate and nano-Si resulted in a 12.1% and 18.5% increase in sesame seed yield, respectively, when compared with control plants. However, the nano-Si application had better positive impacts on crop yield than the calcium silicate (Tab. 3). The higher yield by application of Si fertilizers could be attributed to more significant proline accumulation, higher activity of antioxidant enzymes, and reduction of MDA content. The improvement of seed yield in other crops, such as rice [Kheyri et al. 2019a] and canola [Bukhari et al. 2021], has been observed by the application of Si. Our results are confirmed by Kheyri et al. [2019b], who found that the Si foliar application via either nano-Si or calcium silicate increased the rice grain yield when compared with control plants. Zarei et al. [2020] reported that the combined application of phosphorus nano-chelate and chitosan fertilizers in plants under MDS mitigated the negative impacts of drought stress and improved sesame seed yield.

As shown in Table 3, the Ck-treated plants (1557.1 kg ha^{-1}) indicated significantly higher seed yield over control plants (1454 kg ha^{-1}). Our results are supported by Salek Mearaji et al. [2020], who reported that drought stress reduced the quinoa seed yield compared with standard irrigation treatment, whereas foliar application of Ck (50 μM) increased the seed yield by 23% under regular irrigation and by 13% under drought stress, compared with non-use of Ck. Zaheer et al. [2019] found that the Ck foliar spray increased the wheat grain yield in both optimal irrigation and drought stress.

Malondialdehyde. An excessive amount of reactive oxygen species (ROS) leads to membrane lipid peroxidation, which is a sign of membrane damage under biotic and abiotic stresses [Amirjani and Mahdiyeh 2013]. In our study, the MDA content increased significantly as the drought stress increased. The MDS and SDS led to an increase in MDA content by 21.1% and 38.3%, respectively. The highest increase in the MDA content (57.02 μmol g^{-1} FW) was observed in untreated plants with Si and Ck under SDS (Tab. 4). In similar results, Pourghasemian et al. [2020] reported that the MDA content was increased in response to irrigation treatments, including MDS and SDS. According to Movahhedi Dehnavi et al. [2017], SDS (irrigation after 145 mm evaporation) increased the MDA content by 42.9% compared with MDS (irrigation after 75 mm evaporation).

The results in Table 4 showed that Si application in both calcium silicate and nano-Si forms, as well as the use of Ck, reduced the MDA content in both control and drought stress conditions. Askarnejad et al. [2019] reported that the Si application significantly reduced the amount of damage caused by moisture stress in stevia plants compared with non-application of Si. In similar results, Sepehri and Rouhi [2017] reported that increasing the level of drought stress increased the MDA content, while the application of Ck led to the production of the lowest value of MDA. The simultaneous application of calcium silicate or nano-Si with Ck caused a reduction in the MDA content in both drought stress and control conditions compared with the separate application of Si fertilizers. However, the simultaneous application of nano-Si and Ck under MDS resulted in the production of the lowest value for MDA content (24.66 μmol g^{-1} FW). We also observed that the combined application of nano-Si and Ck reduced the MDA content under MDS and SDS by 7.4% and 6.1%, respectively, compared with nano-Si application only at the same drought stress levels. Kim et al. [2017] stated that the addition of Si to plants leads to a decrease in the MDA content, which is consistent with the results of the present study. It has been documented that the application of Si nanoparticles reduced the adverse effects of water stress by reducing the MDA content [Zahedi et al. 2020].

Proline content. In this study, the proline content in the second year was 11.9% higher than the first year. Our findings showed that the proline content responded significantly to the drought stress. When the plants were subjected to MDS and SDS, the proline content increased by 2.6% and 13.9%, respectively, compared with control plants (Tab. 3). This result indicates a significant increase in the proline content in plants under drought stress, especially under SDS. Proline is one of the most critical osmotic regulators under abiotic stresses, which helps to maintain the membrane's

Table 4. Mean comparison of interactions between drought stress, silicon, and cytokinin on malondialdehyde content in sesame

Drought stress	Control		MDS		SDS	
Treatments	Control	Cytokinin	Control	Cytokinin	Control	Cytokinin
Control	35.18cdef	28.91efgh	44.60b	33.45cdefg	57.02a	38.28bc
Calcium silicate	30.85defgh	29.41defgh	30.59defgh	28.86efgh	35.38cde	36.21cd
Nano-Si	28.28fgh	27.30gh	26.63gh	24.66h	31.86cdefg	29.92defgh

The means with the same letter(s) do not differ statistically by LSD test at $P \leq 0.05$
MDS – moderate drought stress, SDS – severe drought stress

stability and scavenging free radicals [Hoque et al. 2008]. Proline acts as a source of nitrogen and carbon for plants under SDS and increases the plant's tolerance to stress [Amini et al. 2015]. Momeni et al. [2021] demonstrated that the drought stress at 5% field capacity (FC) increased proline content in sesame plants by 42.7% compared with drought stress at 25% FC. The findings in our study that the proline content exhibited a significant increase under drought stress were consistent with Hatamvand et al. [2015], who reported that the proline content in canola significantly increased in response to drought stress at flowering and pod setting stages.

The results presented in Table 3 indicated that the application of both calcium silicate and nano-Si increased the proline content compared with control plants by 10.2% and 18.6%, respectively. However, the nano-Si-treated plants produced significantly higher proline content (0.43 mg g^{-1} FW) when compared with calcium silicate-treated plants (0.39 mg g^{-1} FW). The positive impact of Si in improving the growth of plants under drought stress can be due to the nutritional and antioxidant role of Si for plants [Askarnejad et al. 2019]. The Si application increases the proline concentration by enhancing the cell turgor and water absorption and subsequently improves the plant's resistance to drought stress [Amin et al. 2018]. Kheyri et al. [2018] confirmed that the application of nano-Si facilitates the uptake of Si and other nutrients for plants due to the small diameter of the nanoparticles. Gong et al. [2005] observed that the use of Si caused a significant increase in proline content in the plants under water stress compared with the non-use of Si.

We also observed that the Ck application increased proline content in sesame plants by 11.9% when compared with plants that did not receive Ck. It has been reported that the application of both salicylic acid and kinetin phytohormones enhanced proline content and induced tolerance to drought stress in sesame plants [Hussein et al. 2015]. Maksimovic et al. [2007] mentioned that the Si application mitigated the adverse impacts of drought stress on cucumbers by increasing the antioxidant capacity. Movahhedi Dehnavi et al. [2017] also observed an improvement in proline content at all drought stress levels by foliar application of micronutrients (zinc and boron) compared with control or foliar application of water. Previous studies showed that proline foliar application increased the proline content in quinoa in both drought stress and control treatment [Elewa et al. 2017].

Antioxidant enzyme activity. The results of the combined analysis of variance for antioxidant enzyme activities are shown in Table 5. According to the results, the interactions between DS × Si × Ck were significant in the activities of CAT, APX, and GPX.

CAT activity. The assay of CAT activity revealed that the plots maintained under drought stress had the lowest CAT activity, whereas the plants treated with Ck and Si fertilizers under drought stress significantly showed higher CAT activity. The Ck application increased CAT activity by 25% and 60.7% compared with the non-application of Ck under MDS and SDS, respectively (Tab. 6). Sepehri and Rouhi [2017] found that the application of Ck (150 ppm) increased the CAT activity in plants under drought stress, which is consistent with the results of this study. The CAT activity was significantly higher by combined application of nano-Si and Ck under SDS (0.41) than other experimental treatments. However, there was no significant difference between the simultaneous use of nano-Si

Table 5. Combined analysis of variance for drought stress, cytokinin, and silicon, as well as their interactions on activities of CAT, APX, and GPX in sesame

Source of variation	df	CAT	APX	GPX
Year (Y)	1	0.000003^{ns}	41.03^{**}	3.02^{**}
Replication (R)	4	0.003	0.007	0.0001
Drought stress (DS)	2	0.030^{**}	1.54^{**}	0.03^{**}
Y × DS	2	0.108^{**}	1.31^{**}	0.02^{**}
Error	8	0.0005	0.01	0.0008
Silicon (Si)	2	0.175^{**}	2.71^{**}	0.03^{**}
Y × Si	2	0.017^{**}	2.11^{**}	0.02^{**}
DS × Si	4	0.024^{**}	0.24^{**}	0.01^{**}
Y × DS × Si	4	0.030^{**}	0.23^{**}	0.01^{**}
Cytokinin (Ck)	1	0.115^{**}	2.45^{**}	0.01^{**}
Y × Ck	1	0.037^{**}	1.87^{**}	0.004^{ns}
DS × Ck	2	0.015^{**}	0.09^{*}	0.0005^{ns}
Y × DS × Ck	2	0.006^{ns}	0.10^{*}	0.0004^{ns}
Si × Ck	2	0.0005^{ns}	0.09^{*}	0.006^{*}
Y × Si × Ck	2	0.0004^{ns}	0.10^{*}	0.006^{**}
DS × Si × Ck	4	0.007^{*}	0.06^{*}	0.007^{**}
Y × DS × Si × Ck	4	0.001^{ns}	0.07^{*}	0.007^{**}
Error	60	0.002	0.026	0.001
CV (%)	–	19.25	15.76	19.22
SE	–	0.010	0.075	0.017

^{ns}, *, and ** are non-significant and significant at the 5 and 1% probability levels, respectively CAT – catalase, APX – ascorbate peroxidase, GPX – guaiacol peroxidase

and Ck under MDS (0.37) and the combined application of calcium silicate and Ck under SDS (0.34). We found that the combined application of nano-Si or calcium silicate with Ck had better impacts on CAT activity than the use of Si fertilizers alone. However, the combined application of nano-Si and Ck resulted in 13.5% and 17.1% higher CAT activity than the simultaneous use of calcium silicate and Ck under MDS and SDS, respectively (Tab. 6). It seems that the increase in the antioxidant enzyme activity caused by the foliar application of Ck and Si fertilizers led to the reduction of oxidative stress, scavenging ROS, and increasing tolerance to drought stress in sesame plants. In another study, Bukhari et al. [2021] found that the foliar application of Si at critical growth stages of canola helps to increase the antioxidant enzyme activity and improve the seed yield of canola under drought stress. Likewise, Rahimi et al. [2019] observed that the application of nano-Si increased the activity of antioxidant enzymes of *Calendula officinalis* L. under drought-stress conditions.

APX activity. Our findings indicated that the APX activity displayed an increasing trend with increasing stress levels. The APX activity was increased by 41.5% and 52.5% under MDS and SDS, respectively, compared with the control conditions (Tab. 7). The results of the present research are in line with the findings of Yousefzadeh Najafabadi and Ehsanzadeh [2019], who observed that the APX activity increased by 44% under MDS and by 72% under SDS compared with control irrigation. Momeni et al. [2021] also observed higher APX activity (35.7%) under drought stress at 5% FC compared with 25% FC.

The results showed that the highest APX activity was obtained in the plants treated with Ck and Si fertilizers under MDS. The application of Ck and Si fer-

Table 6. Mean comparison of interactions between drought stress, silicon, and cytokinin on CAT activity in sesame

Drought stress	Control		MDS		SDS	
Treatments	Control	Cytokinin	Control	Cytokinin	Control	Cytokinin
Control	0.22efgh	0.21fgh	0.15hi	0.20gh	0.11i	0.28cdef
Calcium silicate	0.15hi	0.20gh	0.25defg	0.32bcd	0.29cde	0.34abc
Nano-Si	0.29cde	0.32bcd	0.28cdef	0.37ab	0.33bc	0.41a

The means with the same letter(s) do not differ statistically by LSD test at $P \leq 0.05$, CAT – catalase (unit mg^{-1} protein), MDS – moderate drought stress, SDS – severe drought stress

Table 7. Mean comparison of interactions between drought stress, silicon, and cytokinin on APX activity in sesame

Drought stress	Control		MDS		SDS	
Treatments	Control	Cytokinin	Control	Cytokinin	Control	Cytokinin
Control	0.38i	0.77gh	0.65h	0.81fgh	0.80gh	0.80gh
Calcium silicate	0.71gh	1.20cde	1.35bc	1.63a	0.89fgh	1.26cd
Nano-Si	0.72gh	1.07def	1.37abc	1.54ab	0.95efg	1.45abc

The means with the same letter(s) do not differ statistically by LSD test at $P \leq 0.05$
APX – ascorbate peroxidase (unit mg^{-1} protein), MDS – moderate drought stress, SDS – severe drought stress

Table 8. Mean comparison of interactions between drought stress, silicon, and cytokinin on GPX activity in sesame

Drought stress	Control		MDS		SDS	
Treatments	Control	Cytokinin	Control	Cytokinin	Control	Cytokinin
Control	0.11gh	0.15fgh	0.15fgh	0.16efg	0.22bcd	0.15fgh
Calcium silicate	0.17def	0.17def	0.23abc	0.23abc	0.10h	0.18cdef
Nano-Si	0.17def	0.21bcde	0.25ab	0.28a	0.16efg	0.21bcde

The means with the same letter(s) do not differ statistically by LSD test at $P \leq 0.05$
GPX – guaiacol peroxidase (unit mg^{-1} protein), MDS – moderate drought stress, SDS – severe drought stress

tilizers in both control and drought stress conditions increased the APX activity. Under control conditions and SDS, the individual application of nano-Si, calcium silicate, and Ck resulted in similar APX activity, whereas under MDS, nano-Si and calcium silicate had 40% and 40.9% higher APX activity than Ck. However, the increase in APX activity in combined treatments was higher than in individual treatments. The highest APX activity was observed in plants treated by calcium silicate + Ck under MDS (1.63), although there was no significant difference with plants treated by nano-Si alone under MDS (1.37), nano-Si + Ck under MDS (1.54), and nano-Si + Ck under SDS (1.45). The plants treated with calcium silicate + Ck under control conditions (10.8%) and MDS (5.5%) had higher APX activity than plants treated with nano-Si + Ck, whereas under SDS, APX activity by application of nano-Si + Ck (13.1%) was higher than calcium silicate + Ck (Tab. 7). Zhu and Gong [2014] reported that Si nutrition significantly increased the activity of antioxidant enzymes and improved plant growth under water stress. The higher activity of APX under stress conditions results in the scavenging ROS and, as a result, decreases cell death and increases plant resistance to stress [Akhila et al. 2008]. Our results are in agreement with Bukhari et al. [2021], who documented that the Si foliar application can increase the antioxidant enzyme activities and plant tolerance in dealing with environmental stresses. The application of salicylic acid protects plants against the production of ROS and lipid peroxidation by increasing the activity of antioxidant enzymes [Li et al. 2014].

GPX activity. In the present study, drought stress alone increased the GPX activity, as shown in Table 8. A significantly higher GPX activity by 26.7% under MDS and by 50% under SDS was observed compared with control conditions. We found that there was no significant difference in the GPX activity between different levels of drought stress by application of Ck. In contrast, the individual application of calcium silicate and nano-Si led to an increase of 26.1% and 32% in GPX activity under MDS compared with the control treatment. The individual or combined application of Ck with calcium silicate or nano-Si could not improve GPX activity in plants under SDS. GPX activity by separate application of nano-Si and calcium silicate or their simultaneous application with Ck under MDS was significantly higher than similar treatments under SDS and control. The reduction of GPX activity by foliar application of treatments under SDS can be attributed to the elimination of enzyme synthesis [Pourghasemian et al. 2019].

There was no significant difference between the combined application of nano-Si + Ck and calcium silicate + Ck in terms of GPX activity in plants under MDS. However, the activity of GPX in plants under MDS was higher by 17.8% in nano-Si + Ck treatment compared with calcium silicate + Ck. In this research, the highest value for GPX activity was observed in nano-Si + Ck treated plants under MDS (0.28), although there was no significant difference with plants treated by nano-Si alone under MDS (0.25), calcium silicate alone under MDS (0.23), and calcium silicate + Ck under MDS (0.23). This result indicates that both individual application of nano-Si or calcium silicate and their combination with Ck led to higher GPX activity compared with other experimental treatments. The foliar application of phytohormones such as Ck can play an essential role in increasing the activity of drought tolerance mechanisms and improving the growth of sesame plants under water-stress conditions [Mehmood et al. 2021]. Similar to our results, Eisvand et al. [2010] found that the application of Ck could increase the activity of antioxidant enzymes in the plants under drought stress and thus reduce the oxidative stress induced by drought.

CONCLUSIONS

Our findings illustrated that the drought stress, especially the SDS, significantly reduced the sesame seed yield. The application of Ck, calcium silicate, and nano-Si under drought stress, especially under MDS, increased the tolerance of sesame plants to stress. The combined application of nano-Si or calcium silicate with Ck resulted in the reduction of MDA content, more significant accumulation of proline, and higher activity of antioxidant enzymes compared with individual application of Si fertilizers or Ck. However, the nano-Si-treated plants showed an increase in the antioxidant enzyme activities, proline content, and sesame seed yield when compared with calcium silicate-treated plants. Overall, the foliar application of nano-Si + Ck was able to decline MDA content, increase antioxidant enzyme activities, and improve proline accumulation in sesame plants under drought stress, especially under MDS, which helps in improving the sesame productivity under drought stress conditions.

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