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Influence of Foliar Application of Some Biostimulants on Physiological, Agronomic Characters and Crop Water Productivity of Rice Under Water Deficit and Normal Conditions

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WATER deficit is a major environmental stress that has a tremendous effect on the physiological processes and productivity of rice. Thus, it is crucial to enhance rice tolerance to drought stress, especially under unpredictable climate changes and the increasing global population. This investigation aimed to study the impact of foliar-supplied nano-silica (Si-NPs) and potassium sulfate (K-sulphate) on some physiological, agronomic characters and crop water productivity of rice plants under water deficit and well-watered conditions during two summer seasons. The experimental design was a spilt-plot with four replications. The main plots were assigned to the two irrigation treatments; every 4 days (well-watered) and 12 days (water-deficit) after transplanting. The sub-plots were allocated by the three foliar treatments, namely, untreated control (distilled water application), foliar application of Si-NPs, and K-sulphate. The results displayed that the foliar applications, irrigation treatments, and their interactions had a significant influence on all the evaluated traits. Drought stress significantly reduced number of panicles per plant, number of grains per panicle, 1000- grain weight, and grain yield. However, water deficit considerably elevated antioxidant enzymes catalase (CAT) and peroxidase (POD) proline content compared to well-watered conditions. The foliar application of Si-NPs and K-sulphate was efficient in boosting drought tolerance by promoting antioxidant enzymes (CAT and POD) as well as proline accumulation compared with untreated plants. These positive influences were displayed in improving grain yield and its related traits as well as crop water productivity under drought stress conditions. Consequently, the utilization of foliar-applied Si-NPs and K-sulphate represents an applicable approach to enhance yield traits and crop water productivity of rice under water shortage conditions.

Keywords: Rice, Agronomic performance, heatmap, hierarchical clustering, physiological parameters, water deficit.

1. Introduction

Rice (*Oryza Sativa* L.) plays a critical role as a staple cereal crop, meeting the dietary needs of around half of the world's population and contributing to about 6% of their daily calorie consumption (Kobayashi et al., 2023). Its total grown area is approximately 162 million hectares which produces around 765 million tons annually (FAOSTAT, 2023). It is worth emphasizing that a substantial increase of 26% in rice productivity is necessary by 2035 to meet the growing global demand for this essential crop (Ghazi et al., 2023). However, the continued growth of the global population has resulted in an escalating need for rice to meet the rising demand. The development and growth of rice are restricted by the obstacles posed by water deficit conditions (ElShamey et al., 2022). Global climate change has caused significant temperature and precipitation increases. Therefore, climate change leads to water shortage, which is expected to increase in frequency and severity (Qiu et al., 2023). Drought stress is a critical challenge that negatively affects the development and productivity of rice (Sakran et al., 2022). Furthermore, it results in a decisive negative impact on various physiological and biochemical functions (Abd El-Mageed et al., 2021;Morsi et al., 2023). However, plants possess adaptive mechanisms and complex responses that help regulate the impacts

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induced by drought, such as the activation of the antioxidant defense system to neutralize ROS (Farooq et al., 2012;Ali et al., 2021). Proline, an essential non-enzymatic antioxidant, holds a key role in the neutralization of ROS that are produced due to a lack of water (Loudari et al., 2023). Additionally, it strengthens the plant's antioxidant systems and enhances their survival capacity during periods of water scarcity (Desoky et al., 2021). Therefore, it has a primary role in osmotic adjustment in situations of water deficit and in improving drought tolerance. Consequently, it is critical to evaluate effective and environmentally friendly substances to mitigate the negative impacts of drought stress on rice (El-Sanatawy et al., 2021).

Nanobiotechnology has recently gained attention due to its potential to reduce both biotic and environmental stresses (Khan et al., 2017). The use of nanomaterials has yielded some remarkable results in promoting plant development and growth as well as mitigating the negative impacts of various stressors on plants (El-Ramady et al., 2020;El-Temsah et al., 2023). Nanomaterials include silica-nanoparticles (Si-NPs), which are efficient in eliminating the negative impacts of heavy metals, salinity, and ultraviolet radiation (Cui et al., 2017), (Abdel-Haliem et al., 2017;Badawy et al., 2021), (Tripathi et al., 2017). Additionally, SiO2NPs have been shown to mitigate water stress in rice plants (ALVAREZ et al., 2018;Elshayb et al., 2021). Plant leaves absorb nano-silica better than conventional silica because its particle size is below 100 nm (Maghsoudi et al., 2015). Si-NPs accumulation in plant leaves boosts defense enzyme activity and tolerance to ROS (Alsaeedi et al., 2019). Thus, Si-NPs foliar spraying could improve plant drought tolerance (Faizan et al. 2023; Zahedi et al., 2023).

Drought stress tolerance could be enhanced by mineral nutrition. Potassium (K+) is a beneficial macro-element that has a necessary role in organizing multiple physiological and biochemical processes that promote plant resilience against environmental stress such as water deficit (Ahmad et al., 2018). K is a soil-applied mineral; though its absorption by cereal crops remains difficult under conditions of low soil moisture (Wasaya et al., 2021). Consequently, its deficit in the plants can be remedied by exogenous spray which leads to fast absorption and transport in leaf tissues and substantially increased crop yield (Ali et al. 2019). Consequently, the exogenous application of K under drought stress could be an effective approach for increasing crop yield. It has an integral role in preserving cell turgor pressure through osmotic adjustment. Additionally, it is considered valuable in controlling transpiration through stomata closing and opening. Moreover, it improves water relations, antioxidant activity, and defense system activity under drought stress (Zain and Ismail, 2016). Therefore, the K foliage-applied has an enhancing impact on plant growth and productivity under abiotic stresses. The present investigation aimed to study the influence of foliar-supplied nano-silica (Si-NPs) and potassium sulfate (K-sulphate) on some physiological, agronomic traits and crop water productivity of rice plants under water deficit and normal conditions.

2. Materials and Methods

2.1. Experimental location

Feld trial was implemented during the two successive rice summer seasons of 2019 and 2020 at the experimental farm of Sakha Agricultural Research Station, Kafr Elsheikh, Egypt (31°5′ 54″N, 30°57′0″E), to explore the effect of the exogenous application of nano-silica (Si-NPs) and K-sulphate (K_2SO_4) on some more-physiological, and agronomic traits as well as its impact on crop water productivity of rice plants (*Oryza sativa* L., cv. IR-64). Soil samples were collected before cultivation at a depth of 0 to 30 cm and analyzed chemically and physically as outlined by Black *et al*., 1965 (Table 1). The climatic data of two successive rice summer seasons were collected from the Sakha Agro-meteorological station, the central laboratory for agricultural climate, are presented in Table 2

2.2. Experimental design and treatment

The experimental design was a spilt block with four replicates. The main plots were designated to the two irrigation treatments; every 4 days (well-watered) and 12 days (water-deficit) after transplanting. The sub-plots were distributed to the three foliar treatments, namely, untreated control (distilled water application); applied exogenously K-sulfate K_2SO_4 at the rate of 2 ml/L; and nano-silicon at a rate of 0.4 g/L (Sigma Aldrich Chemie GmbH – Taufkirchen - Germany).

Before plowing, the phosphor was added to the nursery at the rate of 50 kg P_2O_5/ha in the form of calcium superphosphate (15.5% P_2O_5). Nitrogen was applied at a rate of 165 kg N/ha in the form of urea (46.0% N) after the last plowing before levelling and shortly before planting. Rice grains were soaked in fresh water for twenty-four hrs and incubated for another forty-eight hrs at a rate of 140 kg/ha. Following that, the seeds was disseminated with 2-3 cm standing water in the nursery in both seasons on April 25. Weeds have been chemically managed at a rate of 4.8 L ha1 with Saturn 50% [S-(4-Chlorophenol methyl) diethyl carbamothioate].

Before plowing, the permanent field received 50 kg P2O5 ha[1]. Seedlings have been carefully retrieved from the nursery 30 days after planting and transplanted onto plots (10 m2) at 20 cm spacing at a rate of 3 seedlings hill1. The herbicide Saturn 50% was applied seven days after transplanting at a rate of 4.8 L/ha. The plots were submerged for two weeks before harvest. The soil was amended with K-sulphate (48% K_2O) at a rate of 60 kg K_2O/ha. During the growth seasons, all other agronomic practices were followed as recommended.

2.3. Studied traits

2.3.1. Proline content

The proline content of leaves was estimated using a technique according to Bates et al. (1973). Briefly, 0.5 g of the largest, most completely developed leaves were powdered with 3% sulfuric acid and spun at 12,000 g for 5 minutes, and the concentration of the resulting solution was determined utilizing ninhydrin reagent. The collected supernatant was homogenized in toluene, and the absorbance at 520 nm was determined using a Shimadzu UV-160A spectrophotometer (Japan).

2.3.2. Determination of enzymatic antioxidant

Catalase (CAT; EC: 1.11.1.6) activity was determined following the procedure described by (Aebi 1984). 100 mM K-phosphate buffer (pH 7.0), a plant extract, and 75 mM H_2O_2 are included in the analysis combination. As a result, the absorbance at 240 nm was calculated employing a UV-visible spectrophotometer. The reaction began using H_2O_2, and the absorbance was measured at 240 nm for 60 seconds. The activity of the CAT enzyme was measured in units of mg1 protein. Furthermore, as mentioned by (Vetter et al. 1958), the peroxidase (POD; EC: 1.11.1.7) activity (mol H_2O_2 min1 g1 FW) was evaluated using o-phenylenediamine as a chromogenic marker in the presence of H2O2 and enzyme extract at 417 nm.

2.3.3. Yield attributes

At harvest time, the number of panicles per hill, the number of grains per panicle, and 1000- grain weight (g) for each plot were determined. Six rows from each plot were harvested and threshed. The grain and straw yields were determined. The yields/ha at 14% moisture content were then computed.

2.3.4. Crop water productivity

Crop water productivity (CWP): was computed according to Quampah et al. 2011 as follows

$$\mathbf{CWP} = \frac{\text{Grain yield (kg/ha)}}{\text{Water consumptive (m}^3\text{/ha)}}$$

2.4. Statistical Analysis

All statistical analyses were performed employing MSTAT computer software package. The averages were compared utilizing Duncan's multiple-range test (Duncan 1955). Moreover, the heatmap was created with the package of RColorBrewer in R software to determine the relationship among studied traits and treatments.

TABLE 1 . Soil physical and chemical properties of the experimental site.

Soil characteristics	2019	2020
Soil texture	Clayey	Clayey
Sand (%)	12.0	12.0
clay (%)	57.0	55.0
Silt (%)	31.0	33.0
EC (dS m^{-1})	3.09	2.98
pH (1: 2.5 water suspension)	8.12	8.17
Organic matter (g kg^{-1})	13.4	13.9
Available P (ppm)	5.70	5.65
Available N (ppm)	58.5	58.0
Available K (ppm)	346	357
Soluble cations (cmolc kg^{-1})		
Mg^{++}	4.40	4.25
Ca^{++}	6.30	6.22
K^+	1.40	1.25
Na^+	19.20	19.0
Soluble anions (cmolc kg^{-1})		
Cl^-	8.80	8.14
HCO_3-	6.50	6.00
CO_3^{--}	0.00	0.00
SO_4^{--}	15.63	15.00

TABLE 2. Air temperature (maximum and minimum, °C) and relative humidity (RH%) at the experimental site during the 2019 and 2020 seasons.

Month	2019			2020		
	Temperature		RH %	Air temperature		RH %
	Max	Min		Max	Min	
May	31.9	25.4	76.4	32.0	23.8	68.9
June	33.1	28.0	81.5	31.1	25.2	78.0
July	33.5	28.4	85.2	33.7	27.3	84.2
August	34.2	28.9	85.7	34.6	28.2	85.3
September	32.4	27.9	83.4	34.6	27.1	86.7
October	30.2	26.7	87.3	31.5	24.6	84.8

3. Results

3.1. Physiological traits

The irrigation treatments, foliar application, and their interactions displayed significant (<0.001) effects on the proline content and the activity of antioxidant enzymes catalase (CAT) and peroxidase (POD) during both seasons of 2019 and 2020 (Table 3). Water deficit caused a substantial increase in proline content by 68.60% and 71.59%, CAT activity by 17.05% and 20.74% and POD activity by 15.03% and 15.82% compared to well-watered plants during both seasons of 2019 and 2020, respectively. The exogenous application of Si-NPs and K-sulphate significantly enhanced proline content, and the activity of antioxidant enzymes compared to untreated plants. The highest proline content and CAT and POD enzymatic activities were obtained using the foliar spray of Si-NPs. It enhanced proline, CAT, and POD by 13.41, 48.59 and 11.65 compared to untreated plants over the two seasons. Likewise, K-sulphate displayed substantial enhancement in proline, CAT, and POD by 10.98 %, 18.24%, and 10.43% respectively compared to untreated control. The interaction values between irrigation treatment and exogenous application were significant during both seasons. The uppermost increase in proline content, CAT and POD activities were observed in the plants exposed to water deficit stress and treated with Si-NPs, whereas the lowest ones were given with untreated plants (control) under normal irrigation in both seasons (Fig. 1).

3.2. Crop Water Productivity (CWP)

As shown in Table 3, irrigation treatments, exogenous applications, and their interactions exhibited significant effects on crop water productivity (CWP). Foliar application of Si-NPs and K-sulphate considerably enhanced CWP in comparison with untreated control. The foliar application of Si-NPs improved CWP by 26.33%. Likewise, K-sulfate enhanced CWP by 7.41% compared to untreated control over both seasons. Interaction effect between irrigation treatment and foliar spray had a considerable effect on CWP. The uppermost CWP was exhibited by exogenous application of Si-NPs followed by K-sulphate under drought stress (Figure 3). Otherwise, the lowermost values were displayed by untreated control under stress conditions in both seasons (Fig. 2).

Table 3. Influence of irrigation treatments and foliar application of nano-silica and K-sulphate on proline content, catalase activity, and peroxidase activity during two summer seasons of 2019 and 2020.

Studied factor		Proline content		Catalase activity		Peroxidase activity		Crop water productivity	
		2019	2020	2019	2020	2019	2020	2019	2020
Irrigation regimes									
Normal irrigation		0.637 **B**	0.697 **B**	0.745 **B**	0.781 **B**	1.610 **B**	1.536 **B**	0.783 **A**	0.700 **A**
Water deficit		1.074 **A**	1.196 **A**	0.872 **A**	0.943 **A**	1.852 **A**	1.779 **A**	0.775 **A**	0.642 **A**
Exogenous application									
Control		0.726 **b**	0.925 **b**	0.675 **b**	0.508 **c**	1.587 **b**	1.539 **b**	0.698 **c**	0.569 **c**
Nano-silica		0.939 **a**	0.965 **a**	1.043 **a**	1.333 **a**	1.795 **a**	1.743 **a**	0.884 **a**	0.832 **a**
K-sulphate		0.900 **a**	0.950 **ab**	0.708 **b**	0.745 **b**	1.770 **a**	1.720 **a**	0.757 **b**	0.612 **b**
ANOVA	**df**	***P* value**							
Irrigation (Ir)	1	< 0.001	< 0.001	0.017	0.004	< 0.001	0.002	0.528	0.371
Application (A)	2	< 0.001	0.021	0.004	< 0.001	< 0.001	< 0.001	0.017	0.008
Ir×A	2	< 0.001	< 0.001	0.001	< 0.001	< 0.001	0.001	0.001	< 0.001

Averages followed by same letters under the same studied factor do not differ significantly by Duncan's multiple-range test at $p \leq 0.05$.

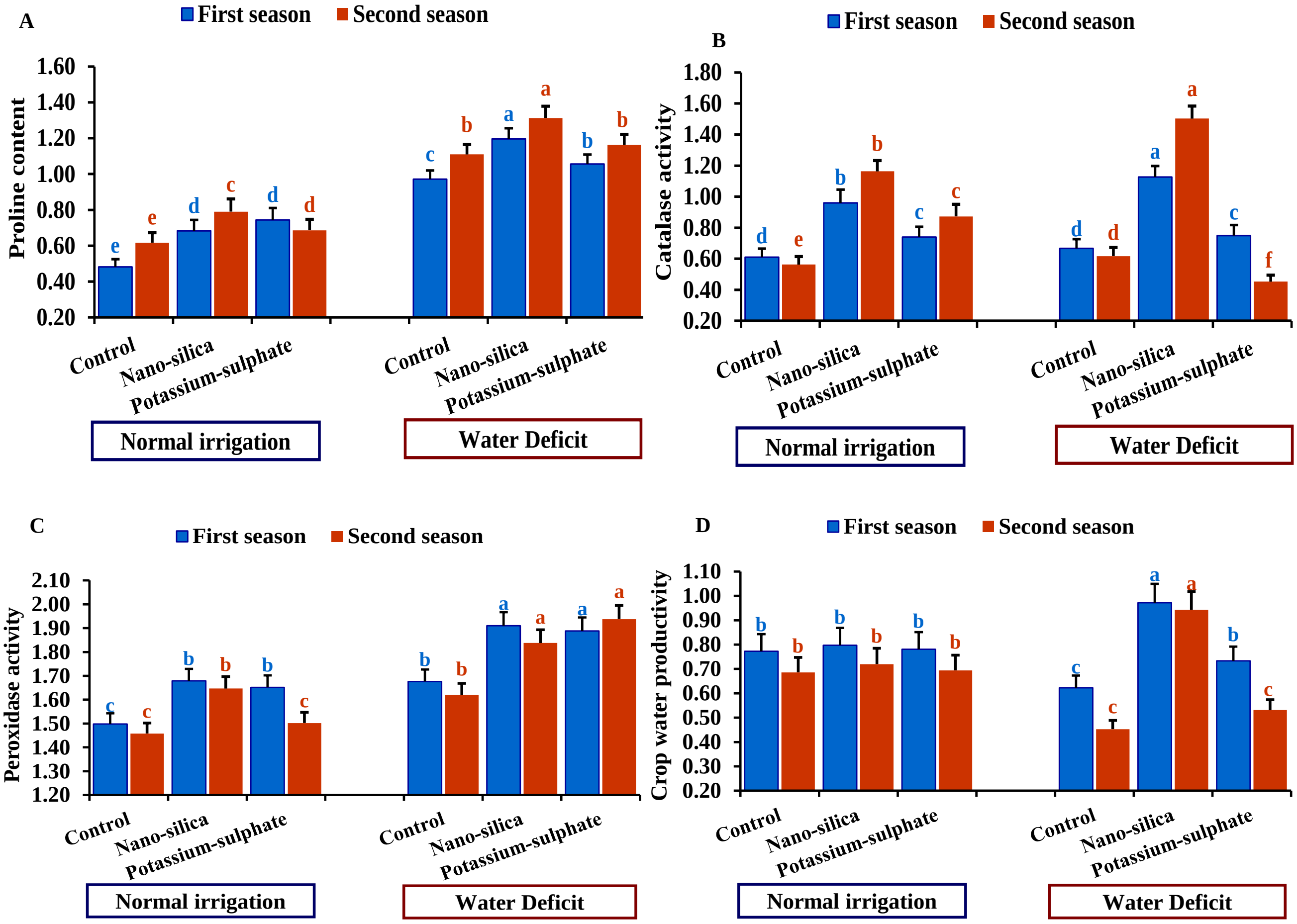

Fig. 1. Impact of interaction effect between irrigation regimes and foliar application of nano-silica and K-sulphate on proline content, catalase activity, peroxidase activity and crop water productivity for two summer seasons of 2019 and 2020.

4. Yield traits

Grain yield and contributing traits were considerably affected by the irrigation treatments in both seasons. Rice plants induced by drought stress exhibited lower yield traits than well-watered plants (Table 4). Drought stress led to a significant reduction in number of panicles/plant by 26.83 and 25.86%, number of grains per panicle by 25.28 and 12.42%, 1000- grain weight by 12.85 and 13.06%, and grain yield by 20.37 and 25.66% in both seasons 2019 and 2020 respectively. However, the application of Si-NPs and K- sulphate minimized the reduction in these traits when plants were induced by drought stress. The foliar application alleviated the negative effects of drought stress compared with untreated control. Plants treated by both applications exhibited considerable enhancement in grain yield and contributing traits. The applied Si-NPs improved number of panicles per plant, number of grains per panicle, 1000- grain weight, and grain yield by 5.7%, 8.6%, 2.2%, and 21.5%, respectively, compared with untreated plants (Table 4). Similarly, K-sulphate boosted the aforementioned traits in the same order by 6.2%, 1.3%, 2.32%, and 5.22% compared to untreated control (Table 4). The interaction effects between irrigation and foliar treatments had a considerable impact on number of panicles per plant, number of grains/panicle, 1000- grain weight, and grain yield. The maximum values of these traits were recorded in adequately irrigated rice plants that were treated with foliar spraying with Si-NPs and K-sulphate in both seasons, whereas the lowermost ones were assigned for untreated control under drought stress in both seasons (Fig. 2).

Table 4. Influence of irrigation treatments and foliar application of nano-silica and K-sulphate on grain yield and contributing traits during two summer seasons of 2020 and 2021.

Studied factor		No of panicles per plant		No of grains per panicle		1000- grain weight		Grain yield	
		2019	2020	2019	2020	2019	2020	2019	2020
Irrigation regimes									
Normal irrigation		25.53 **A**	23.78 **A**	136.1 **A**	135.3 **A**	27.55 **A**	25.73 **A**	11.28 **A**	10.08 **A**
Water deficit		18.68 **B**	17.63 **B**	101.7 **B**	118.5 **B**	24.01 **B**	22.37 **B**	7.11 **B**	5.89 **B**
Exogenous application									
Control		21.14 **b**	19.93 **b**	114.7 **b**	123.3 **b**	25.57 **a**	23.52 **b**	8.42 **b**	7.07 **b**
Nano-silica		22.17 **ab**	21.36 **a**	127.4 **a**	133.0 **a**	25.81 **a**	24.37 **a**	10.19 **a**	9.51 **a**
K-sulphate		23.00 **a**	20.83 **ab**	114.50**b**	124.5 **b**	25.96 **a**	24.28 **a**	8.98 **b**	7.38 **b**
ANOVA	**df**	***P* value**							
Irrigation (Ir)	1	< 0.001	0.006	< 0.001	0.002	0.012	0.002	< 0.001	0.004
Application (A)	2	0.008	0.018	0.003	< 0.001	0.817	0.016	0.003	< 0.001
Ir×A	2	0.003	0.021	0.022	< 0.001	0.007	0.429	0.002	< 0.001

Averages followed by same letters under the same studied factor do not differ significantly by Duncan's multiple-range test at p ≤ 0.05.

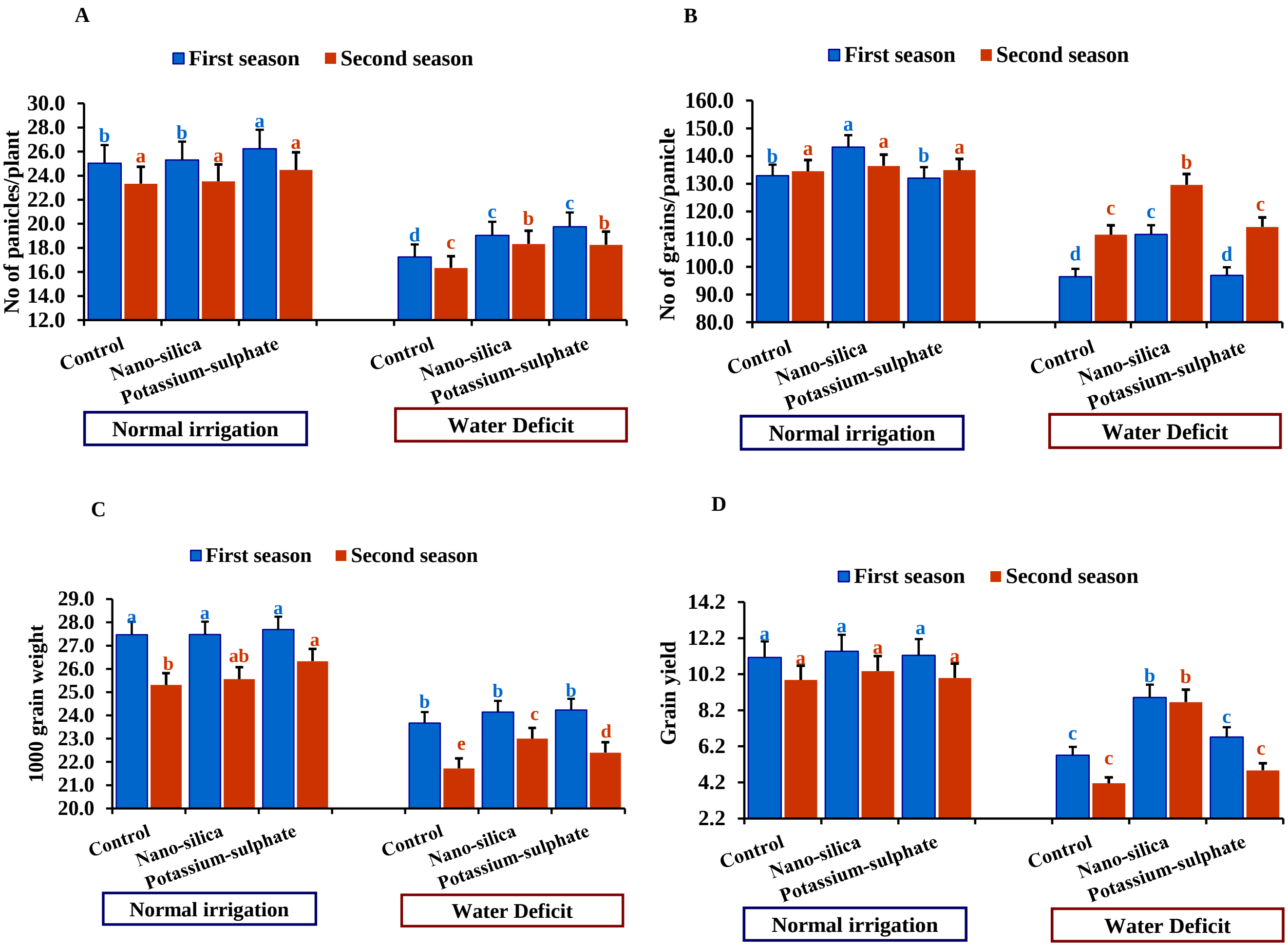

Fig. 2. Number of panicles plant (A), number of grains per panicle (B), 1000- grain weight (C), and grain yield (D) as impacted by the interaction between irrigation treatments and foliar application of nano-silica and K-sulphate during two summer seasons of 2019 and 2020.

Heatmap and hierarchical clustering

The analysis of Heatmap was performed based on the evaluated traits. The irrigation regimes and foliar applications were separated into distinct clusters (Fig. 3). Irrigation was the primary separating factor of the clusters. The application of Si-NPs followed by K-sulphate exhibited the greatest values (described in blue) for most studied traits under well-watered and drought stress conditions. Otherwise, the untreated control displayed the lowest values (described in red) for all evaluated traits, particularly under water deficit. Moreover, the dendrogram between dependent variables displayed that all studied variables were grouped into two different clusters (Fig. 3). The first cluster included proline content, POX activity, CAT activity, and CWP. While the second cluster included number of panicles/plant, number of grains/panicle, 1000- grain weight, and grain yield

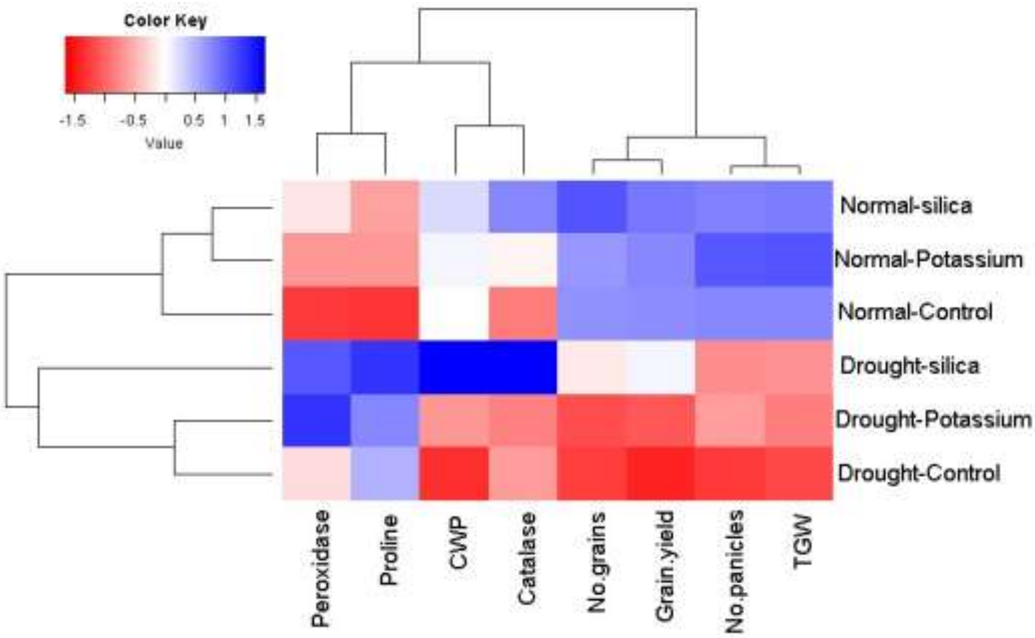

Fig. 3. Heatmap separating the assessed exogenous application and irrigation treatments into distinct groups based on physiological and agronomic characters. Red and blue colours reveal low and high.

4. Discussion

Drought is a significant environmental stress that has adverse effects on rice development and productivity (Abd EL-Aty et al. 2023). As a result, developing effective and long-term solutions to attenuate the harmful impacts of water deficit has become more important, especially considering present climatic variability (Mansour et al., 2023). This work explored the effectiveness of applied Si-NPs and K-sulphate in alleviating the negative effects of water shortage in rice. The obtained results displayed that the irrigation treatments, foliar applications, and their interactions had a significant influence on all the evaluated parameters. Drought stress considerably reduced number of panicles/plant, 1000- grain weight, number of grains/panicle, and grain yield. Otherwise, antioxidant enzymes and proline content were raised compared to non-stressed conditions. These results were in harmony with previously published studies that confirmed the negative impact of drought stress on the numerous physiological and agronomic characteristics of rice (Panda et al., 2021;Hussain et al., 2022;Moukoumbi et al., 2023).

Proline is classified as a proteinogenic amino acid, which has an ability to accumulate in response to various environmental stressors (Soares et al., 2019;Ullah et al., 2023). It has an important role against environmental stresses through scavenging free radicals and enhancing enzyme activity. In the current study, water shortage stress considerably elevated the amount of proline in water-stressed plants compared to those of normal conditions. Furthermore, the foliar sprays of Si-NPs and K-sulphate significantly enhanced proline content under water deficit compared with untreated control. Applied Si-NPs and K-sulphate resulted in an augmentation of plant defense mechanisms through the modulation of enzyme activity and proline content compared with stressed and untreated rice plants. This adjustment facilitated ROS scavenging and provided protection against oxidative damage in the cells of rice plants subjected to drought stress These findings are in harmony with those provided by (Abdel-Haliem et al., 2017;Mathur and Roy, 2020;Tortella et al., 2023).

The accumulation of antioxidant components by plants under stress conditions is a significant defense mechanism against drought stress (Elsherpiny et al. 2023), which protects plant cells from negative impacts of drought stress and preserves membrane integrity (Hegazy et al., 2015;Desoky et al., 2023). There is a considerable association between tolerance against environmental stress and increased antioxidant activity. The results of this study exhibited that the antioxidant enzymatic CAT and POD activity were substantially elevated in the stressed rice plants compared to the unstressed control. Moreover, the application of Si-NPs and K-sulphate significantly boosted the antioxidant system by simulating the activity of CAT and POD compared to untreated plants under drought stress. The improvement of enzyme activity boosted rice plants' ability to defend against oxidative impairment caused by water deficit and hence enhanced tolerance to drought stress. Foliar application of nano-silica had the greatest values when compared to the other treatments. Consequently, the application of Si-NPs could serve as a useful tool for increasing rice's tolerance to oxidative stress by bolstering its enzymatic defences against ROS. In this regard, (Mukarram et al., 2022) (El-Shawa et al., 2022) (Elshayb et al., 2021) reported that the use of Si-NPs increased antioxidant enzymes against oxidative stress.

Grain yield is the ultimate product of the physiological processes of different stages of plant development. The obtained results pointed out that water deficiency conditions significantly declined grain yield and its contributing traits, comprising number of grains/panicle, number of panicles/plant, and 1000- grain weight compared to those under normal conditions. This reduction was due to the decline in nutrient assimilation and transfer over grain ripeness. In this context, it has been stated that the water deficit caused a substantial decrease in the absorption of plant nutrients. Consequently, the plant experienced inadequate nourishment, which likely resulted in a reduction in 1000- grain weight, number of grains/panicle, and finally grain yield. On the contrary, agronomic traits were significantly boosted by the application of Si-NPs and K-sulphate in the treated plants under drought stress compared with untreated stressed control. The best performance was possessed by the treated plants with Si-NPs compared to other treatment. These findings might be attributed to the fact that Si-NPs act as a growth regulator in rice plants, increasing their resistance to water scarcity by boosting water absorption, mineral uptake, and scavenging ROS. In this context, Saad Kheir et al. (2019);Elshayb et al. (2021) disclosed that the exogenous application of Si-NPs enhanced grain yield and contributing traits under abiotic stress conditions.

5. Conclusions

Water deficit stress substantially reduced number of grains/panicle, number of panicles/plant, 1000- grain weight, and grain yield. Otherwise, it greatly elevated the activity of antioxidant enzymes (CAT and POD) and proline content compared to well-watered control. The exogenous application of Si-NPs and K-sulphate is an effective method to alleviate the adverse effects caused by drought stress by enhancing enzymatic antioxidants and proline content. These improvements were reflected in the enhanced crop water productivity and rice production. Hence, foliar application of Si-NPs and K-sulphate could be exploited as an effective method to boost tolerance against drought stress in rice under water scarcity conditions.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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