

Original Research

Effect of Some Biostimulants on Agronomic, Physiological, and Quality Traits of Wheat Plants (*Triticum aestivum* L.) under Water Deficit Stress Conditions

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Abstract

Two field experiments were performed to investigate the effect of foliar application on the productivity of bread wheat cultivars under water deficit stress conditions. The experiment focused on three bread wheat cultivars (Sakha 95, Giza 171, and Misr 1), comparing typical irrigation practices (four irrigations) against water-stressed conditions (one irrigation). Six foliar spray treatments were included in the study. The foliar applications were control, Seaweed, Ascobin, Lithovit with boron, Lithovit with nitrogen, and Cina green plus. The seasons, water treatments, and foliar spray had significant differences for most studied characters. The means of the studied traits in the 2018/19 season were significantly greater than those in 2017/18, and all studied characters significantly decreased under water stress conditions. Among the foliar applications, Cina green plus and Lithovit with nitrogen consistently yielded the most favorable results for several key indicators, including relative water content, catalase and peroxidase activity, as well as proline content. These findings were observed under both normal and water stress conditions. The study suggests that foliar applications of Cina green plus and Lithovit with nitrogen have the potential to enhance the growth and yield characteristics of wheat crops, even under drought-stress

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conditions. These applications might serve as potential agricultural fertilizers, mitigating the negative effects of water deficit stress on wheat plants.

Keywords: bread wheat, ascorbin, biofertilizer, lithovit, water deficit stress

Introduction

Wheat has a prominent position among the most crucial and extensively grown crops. There are many environmental factors limiting crop productivity, such as salinity [1–4], heat [5, 6], and water deficit stress [7, 8]. Limited water availability, known as water stress, ranks among the most impactful environmental factors hindering plant growth and development [9, 10]. Further, climate change is a growing threat to agriculture and ecosystems worldwide [11]. Agronomic, physiological, and grain quality changes are caused by water stress in wheat. When water stress occurs, agronomic and morphological characteristics generally decrease [12, 13]. Conversely, proline contents, catalase, and peroxidase activity were found to increase under water stress [14].

The application of biostimulants is a very important new approach in crop fertilization to minimize the harmful effects of chemical fertilizers and keep the environment safe. Biostimulants are important substances that can stimulate the growth and development of plants even when used in small amounts and have been studied for their potential benefits in horticultural and agricultural crops [15]. Future research should prioritize the evaluation of biostimulant effectiveness on cereal crop yields. Seaweed extracts used as biostimulants boast a diverse range of active ingredients, including growth-promoting hormones like cytokinins, polyamines, and brassinosteroids [16]. Studies show that using algal extracts can improve plant growth and increase crop yields [17]. Previous studies indicated that applying active compounds extracted from algae can mitigate the negative effects of water stress [18] and nutrient deficiencies [19] in several crops. Additionally, finely ground limestone, known as Lithovit, is another type of biostimulant. This material is primarily composed of calcium and magnesium carbonates (Ca , Mg-CO_3) and contains various essential plant nutrients (Patent DE202006011165 U1). The application of Lithovit to wheat plants has been shown to improve growth and yield. This compound contains Mg , Ca , Fe , and Si , which are thought to increase chlorophyll pigments, resulting in improved water content of plants under stress [20]. Further studies have shown that Lithovit can also enhance photosynthesis by elevating carbon dioxide (CO_2) concentrations within the leaf's internal spaces (intercellular spaces) [21]. Additionally, it has been demonstrated to enhance the chlorophyll content and dry matter of tomatoes under salinity conditions [22]. Ascorbin treatment demonstrably promoted the growth and production of active compounds in plants, even under stressful conditions [23]. Moreover,

since ascorbic acid has been shown to stimulate antioxidant defense for enhanced drought resistance, this study investigated the potential of Seaweed extract, Lithovit, Ascorbin, and Cina green plus biostimulants to improve wheat performance under both normal and water-stressed conditions associated with agronomic, physiological, and quality traits.

Materials and Methods

This study was undertaken at the Faculty of Agriculture's experimental farm, Kafrelsheikh University, during the 2017/18 and 2018/19 seasons. The location sits at 31°07' N latitude, 30°57' E longitude, and approximately 6 m above sea level. In each season, the experiments were separately performed under two water treatments. In the first water treatment (normal), five irrigations were applied, while in the first water treatment (stress), only the planting irrigation was applied. The experiment employed a split-plot design with three replicates under each water treatment. The researchers randomly assigned the studied cultivars to the main plots, while the subplots received the various foliar spray treatments. Each subplot measured 4.2 m², consisting of six rows spaced 20 cm apart and measuring 3.5 m long. Sowing occurred on November 25th and 27th in both the 2017/2018 and 2018/2019 growing seasons.

Three commercial bread wheat cultivars were used, and their names, pedigree, selection history, and origin are shown in Table 1. In addition, six foliar spray treatments were applied, and their name, composition, and concentration are shown in Table 2. All these compounds were applied three times with the concentration of 1.5 g L⁻¹ as a foliar application at 40, 50, and 60 days after planting.

Standard agricultural practices, excluding irrigation, were applied following recommended guidelines to meet the requirements of the plants. The preceding crops were maize and rice, planted in the first and second seasons, respectively. Table 3 presents the average monthly air temperature (°C) and rainfall (mm/month) experienced during the growing season at the experimental site.

The samples were collected at 65 days from sowing to determine leaf indices and physiological characteristics; however, the plant height and other characteristics in seeds were recorded at the harvest date. The following characteristics were studied: plant height (cm), no. of spikes m⁻², weight of 1000 kernels (g), grain yield plant⁻¹ (g), relative water content (%) [24], activity of peroxidase and catalase ($\mu\text{mol min}^{-1} \text{g protein}^{-1}$) [25], leaf proline content (mg g⁻¹ FW) [26], grain protein content (%) [27],

Table 1. The investigated bread wheat genotypes pedigree and selection history.

Genotypes	Pedigree	Selection history	Origin
Sakha 95	PASTOR // SITE / MO /3/ CHEN / AEGILOPS SQUARRO-SA (TAUS) // BCN /4/ WBLL1.	CMA01Y00158S-040POY-040M-030ZTM-040SY-26M-0Y-0SY-0S	Egypt
Giza 171	SAKHA 93/GEMMEIZA 9	GZ2003-101-1GZ-4GZ-1GZ-2GZ-0GZ	Egypt
Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR	CMSS00Y01881T-050M-030Y-030M030WGY-33M-0Y-0S	Egypt

Table 2. The names and concentrations of the foliar spray materials.

Name	Composition	Concentration
Control	Tap water	-
Seaweeds	16% alginic acid, 1.6% mannitol, 0.6–2% N, 1.3% P ₂ O ₅ , 18–20% K ₂ O, 1.5–3% micronutrients, and 45–55% organic matter	200 g fed ⁻¹
Lithovit with boron	3% chloride calcium, 2% chloride magnesium, 1.13% boric acid, 0.13% ferrous sulfate, 20% calcium carbonate, 35% magnesium carbonate	250 g fed ⁻¹
Ascobin	Ascorbic acid and citric acid in a ratio of 2:1	200 g fed ⁻¹
Lithovit with nitrogen	25% urea, 3% chloride calcium, 2% chloride magnesium, 5% calcium carbonate, 7.8% magnesium carbonate	500 g fed ⁻¹
Cina green plus	20% N, 20% P, 20% K, 1000 ppm Fe, 700 ppm Zn, 7000 ppm Mn	500 g fed ⁻¹

Table 3. The average monthly air temperature (°C), RH%, and rainfall (mm/month) experienced during the growing seasons.

Month	AT°C 2017/18		AT°C 2018/19		RH%		Rainfall (mm)	
	Max.*	Min.**	Max.	Min.	2017/18	2018/19	2017/18	2018/19
November	25.3	13.4	27.0	15.2	62.2	57.6	30.0	10.1
December	22.0	11.5	21.0	10.7	68.1	63.9	4.1	12.5
January	19.7	8.9	19.3	6.7	67.9	53.0	29.7	6.1
February	23.2	10.3	21.4	7.8	60.5	57.0	5.6	6.7
March	29.3	12.1	24.0	9.5	44.2	54.8	1.8	16.7
April	31.5	14.3	28.2	12.4	43.4	47.3	11.5	3.0
May	36.1	19.2	36.7	17.4	40.8	34.1	0.0	0.0

wet gluten content, and germination percentage [28]. A combined analysis was conducted across both water treatments and seasons [29]. Seasons were considered random effects, while water and foliar spray treatments and cultivars were considered fixed effects. Data analysis was conducted via statistical software packages, including Microsoft Excel 2016 and GenStat 18 [30].

Results

Table 4 displays the average values (means) and variations (mean squares) observed for the investigated traits across both seasons, categorized by water

treatment and foliar spray treatment. The seasons, water treatments, and foliar spray had substantial differences for the investigated characters, except for protein content due to foliar spray. In addition, the mean squares were significant for relative water content due to seasons×water treatments, no. of spikes, grain yield, catalase activity, and gluten content due to seasons×cultivars, proline, chlorophyll content and gluten contents due water treatments×foliar spray applications, gluten content due to cultivars×foliar spray applications, relative water and gluten contents due to seasons×water treatments×cultivars and gluten content due to water cultivars×cultivars×foliar spray applications. Moreover, water treatments×cultivars interaction means squares significantly impacted all studied characteristics,

Table 4. Mean squares and the mean performance of the studied parameters under water treatments, cultivars, and foliar spray application in the 2017/18 and 2018/19 seasons.

Treatments	PH	SM	KW	GY	RWC	CAT	POX	PROL	Chl	PROT	Germ	GLU
Season (S)	136.17*	260.04	13.21**	0.089**	104.3**	0.035**	1.631**	0.005**	38.4**	5.03**	168.86**	74.72**
2017/18	114.75	358.73	55.01	0.872	88.47	0.483	2.032	0.422	13.40	12.66	89.78	28.87
2018/19	116.34	360.93	55.51	0.913	89.86	0.458	1.858	0.412	14.25	12.97	91.55	30.04
Water treatments (W)	1298.01**	9269.56**	1462.23**	0.229**	245.43**	0.039**	0.907**	0.172**	111.08**	20.95**	1005.44**	195.21**
Normal	118.00	366.38	57.86	0.925	90.23	0.457	1.880	0.389	14.54	13.12	92.83	30.40
Water stress	113.09	353.28	52.66	0.860	88.10	0.484	2.010	0.445	13.11	12.50	88.51	28.50
Cultivars (C)	1419.29**	1900.34**	616.17**	0.715**	93.95**	0.05**	3.543**	0.026**	93.504**	4.26**	108**	31.75**
Sakha 95	120.35a	365.36a	56.71b	1.005a	89.06b	0.474b	1.954b	0.436a	12.68c	13a	92.03a	28.84c
Giza 171	114.69b	355.21c	57.18a	0.859b	90.36a	0.443c	1.719c	0.399c	13.83b	12.9a	90.33b	29.36b
Misir 1	111.59c	358.92b	51.89c	0.814c	88.08c	0.496a	2.162a	0.416b	14.96a	12.54b	89.65c	30.16a
Foliar spray treatments (Sp)	105.63**	983.56**	30.13**	0.025**	10.82**	0.012**	0.158**	0.004**	2.14**	0.17	7.99**	15.85**
Control	114.06c	353.89c	54.55d	0.856d	88.29c	0.443c	1.827c	0.4c	13.42c	12.68a	89.74b	28.31e
Seaweed	116.69ab	358.61bc	54.92cd	0.882c	89.05b	0.467b	1.941b	0.415b	13.84b	12.81a	90.88a	29.57c
Lithovit with boron	116.56b	361.22b	55.07cd	0.884c	89.09b	0.466b	1.933b	0.419b	13.82b	12.86a	90.75a	29.66bc
Ascobin	116b	364.28ab	55.26c	0.889c	89.18b	0.467b	1.964b	0.416b	13.79b	12.81a	90.71a	29.11d
Lithovit with nitrogen	116.22b	359.67b	56.32b	0.914b	89.42b	0.486a	1.984ab	0.421b	13.92b	12.83a	91.04a	29.89b
Cina green plus	118.5a	367.89a	56.93a	0.93a	89.98a	0.496a	2.022a	0.432a	14.17a	12.88a	90.9a	30.18a
S x W	0.07	17.23	0.08	0.0001	3.96*	0.0002	0.009	0.001	0.05	0.06	0.17	0.04
S x C	3.85	269.01*	0.29	0.053**	0.57	0.005**	0.033	0.0003	0.15	0.34	4.3	5.49**
S x Sp	1.5	0.1	0.17	0.0002	0.61	0.0001	0.001	0.00002	0.12	0.02	0.17	0.05
W x C	51.72*	147.45	56.99**	0.003	6.75**	0.003**	0.179**	0.013**	1.72**	2.33**	5.68*	12.76**
W x Sp	1.002	6.17	0.8	0.0002	0.22	0.00001	0.005	0.001*	2.07**	0.02	0.02	3.86**



C x Sp	3.31	5.18	0.86	0.001	0.08	0.0001	0.002	0.0004	0.12	0.02	0.2	3.29**
S x W x C	0.02	94.53	0.002	0.002	2.82**	0.0001	0.003	0.001	0.04	0.04	1.29	3.45**
S x W x Sp	1.24	6.7	0.08	0.001	0.12	0.00002	0.003	0.00005	0.02	0.01	0.02	0.24
S x C x Sp	1.3	7.99	0.08	0.0003	0.04	0.0001	0.001	0.00002	0.07	0.01	0.06	0.23
W x C x Sp	4.23	15.79	0.6	0.001	0.29	0.0001	0.007	0.0004	0.13	0.02	0.03	2.58**
S x W x C x Sp	0.95	12.33	0.01	0.001	0.21	0.0001	0.002	0.00004	0.11	0.02	0.03	0.17
CV	3.24	2.45	1.44	4.68	0.91	6.68	6.39	3.52	3.63	3.17	0.98	2.15

Season (S), Water treatments (W), Cultivars (C), and Foliar spray treatments (Sp).

* and ** NS indicate $P < 0.05$ and $P < 0.01$, respectively. PH=plant height (cm), SM=no. of spikes m^{-2} , KW=1000-kernel weight, GY=grain yield $kg\ m^{-2}$, RWC=relative water content%, CAT=catalase activity ($\mu mol\ min^{-1}\ g\ protein^{-1}$), POX=peroxidase activity ($\mu mol\ min^{-1}\ g\ protein^{-1}$), PROT=protein content%, Ger=Germination% and GLU=wet gluten content.

with the exception of grain yield. Averaged across all studied conditions, the means of the studied traits in 2018/19 (Table 4) were significantly greater than those in 2017/18, except for catalase and peroxidase activity as well as proline contents which were vice versa.

The presented data in Table 4 showed catalase, peroxidase activity, and proline content across both seasons and when considering all cultivars and foliar spray applications. The study found that water stress significantly reduced all the investigated plant characteristics. Across all seasons, water treatments, and foliar spray applications, the Sakha 95 consistently demonstrated superior performance in several key growth and yield parameters. These included plant height, no. of spikes m^{-2} , grain yield, proline content, protein content, and germination percentage. Furthermore, the Giza 171 cultivar excelled in terms of both the weight of 1000 kernels and the relative water content.

Moreover, Misr 1 showed the highest values of catalase content, peroxidase content, chlorophyll content, and gluten content. In contrast, Misr 1 consistently recorded the lowest values in terms of plant height, 1000-kernel weight, grain yield, relative water content, chlorophyll content, protein content, and germination%. Additionally, Giza 171 showed the lowest values of no. of spikes m^{-2} , catalase content, peroxidase content, and proline content. Furthermore, Sakha 95 showed the highest values of chlorophyll content and gluten content.

Compared to the control, Cina green plus treatment differed significantly and showed the highest values of plant height, relative water content, proline content, chlorophyll content, 1000-kernel weight, and gluten content. In addition, Cina green plus and Ascobin applications differed significantly and showed the highest values of no. of spikes m^{-2} . Moreover, Cina green plus and Lithovit with nitrogen application differed significantly and showed the highest values of catalase content. Furthermore, the foliar spray treatments differed significantly from the control and achieved the greatest value of germination%. Conversely, the foliar spray treatments did not differ significantly in protein content.

Interaction Effect

Plant Height (cm)

The findings presented in Fig. 1 indicate that Cina green plus generally resulted in the tallest plants across various conditions. Conversely, the control treatment consistently yielded the shortest plants. Additionally, for the Giza 171, applying Lithovit with boron in the 2018/19 season (under both normal and water stress conditions) led to the tallest plants. Furthermore, Seaweed application proved effective in promoting taller plants: for Sakha 95 under water stress, Giza 171 under normal irrigation in 2017/18, and Misr 1 under normal irrigation in 2018/19.

no. of Spikes m^{-2}

Fig. 2 illustrates that Cina green plus, applied as a foliar spray, yielded the greatest no. of spikes m^{-2} under most

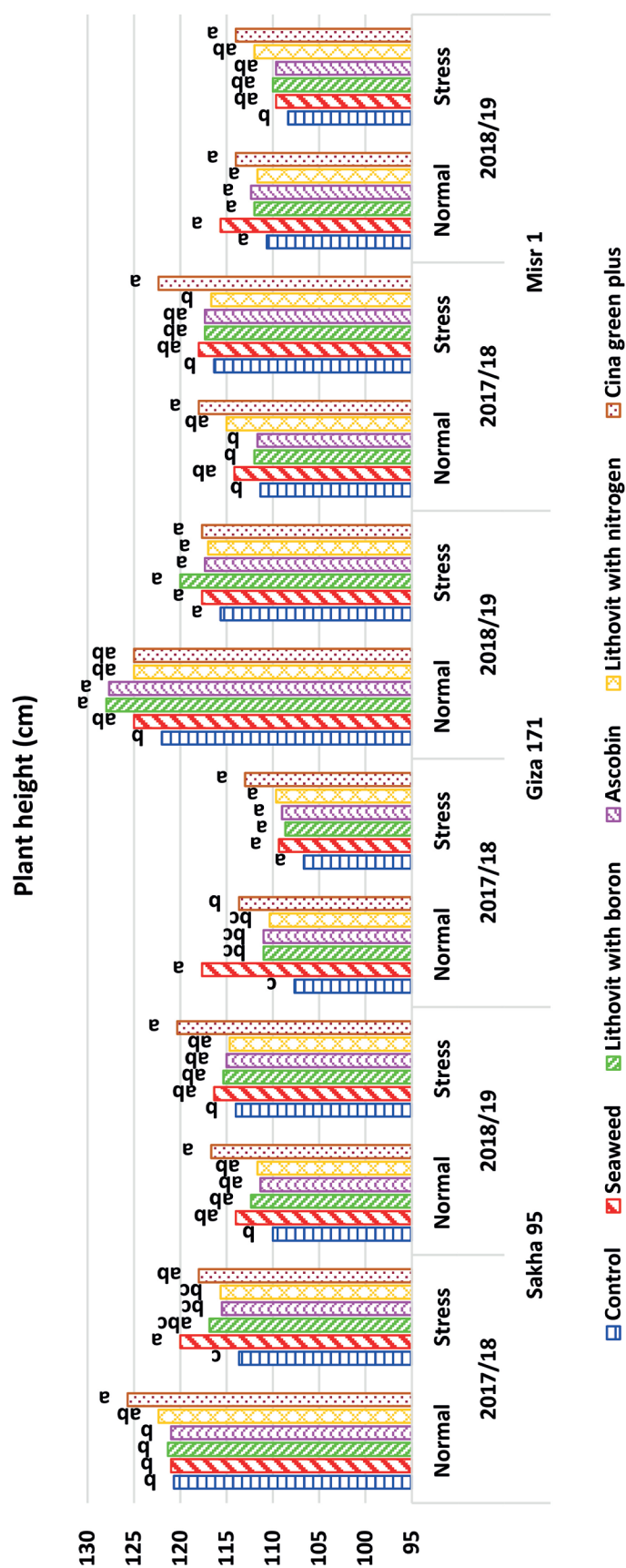
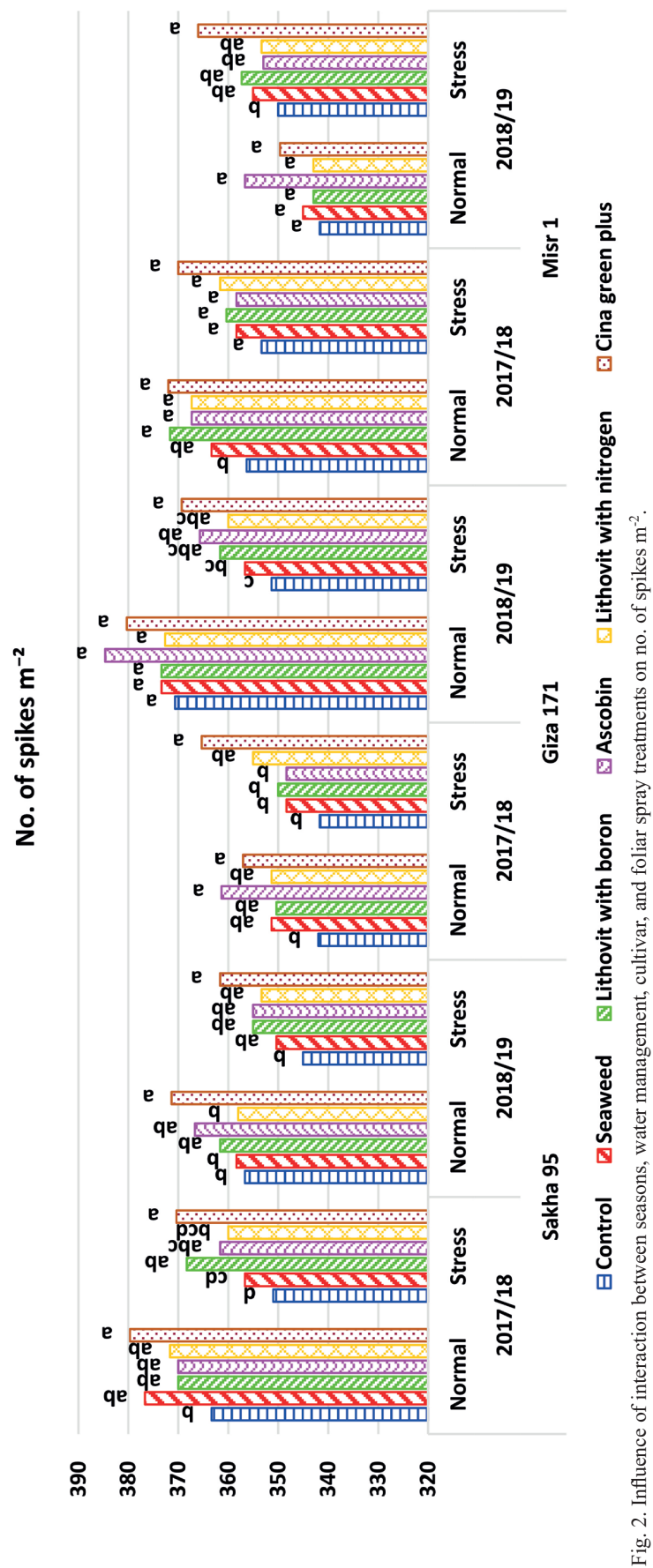


Fig. 1. Influence of interaction between seasons, water management, cultivar, and foliar spray treatments on plant height.



conditions, outperforming the control treatment. In other instances, Ascobin spray proved most effective in increasing the no. of spikes m^{-2} . This was observed under normal conditions for the Giza 171 cultivar in both seasons and for Misr 1 in the second season. Notably, the control treatment consistently resulted in the lowest no. of spikes m^{-2} .

1000-Kernel Weight (g)

The weight of 1000 kernels in Fig. 3 reached its highest value by the foliar application of Cina green plus under most conditions. The control treatment, in contrast, had the lowest values. Similarly, spraying using Lithovit with nitrogen produced the highest values for Sakha 95 under water stress conditions in the 2018/19 season, Giza 171 under normal conditions in the 2018/19 season, and Misr 1 under water stress conditions in the 2017/18 season.

Grain Yield (kg m^{-2})

According to Fig. 4, applying Cina green plus as a foliar spray consistently led to the highest grain yields across various conditions. Contrary to this, the control scored the lowest estimates. In addition, Lithovit with nitrogen showed the highest values under water stress conditions for Sakha 95 in the 2017/18 season and Giza 171 in the 2018/19 season. Moreover, Lithovit with boron produced the highest estimates for Misr 1 under water stress conditions in the first season.

Relative Water Content (%)

Fig. 5 reveals that the foliar application of Cina green plus resulted in the highest levels of relative water content among the treatments. Conversely, the control value was the lowest. Furthermore, applying ascobin as a foliar spray during normal irrigation resulted in the most favorable results for the Sakha 95 cultivar in the first season and the Giza 171 cultivar in the second season.

Catalase Activity ($\mu\text{mol min}^{-1} \text{g protein}^{-1}$)

As illustrated in Fig. 6, Cina green plus applied as a foliar spray consistently yielded the highest catalase activity across various conditions, while the control treatment consistently yielded the lowest. Lithovit with nitrogen, on the other hand, proved most effective in boosting catalase activity for Sakha 95 under normal conditions in the first season and water stress in the second season and for Misr 1 under all irrigation conditions in the first season and water stress in the second season.

Peroxidase Activity ($\mu\text{mol min}^{-1} \text{g protein}^{-1}$)

Fig. 7 reveals that Cina green plus, applied as a foliar spray, consistently yielded the highest peroxidase activity across most conditions, while the control treatment consistently yielded the lowest. Among the other treatments, Lithovit nitrogen was most effective for Sakha 95 under

water stress in the second season. Additionally with, Lithovit with boron led to the highest activity for Giza 171 under water stress in the first season. Furthermore, applying Seaweed as a foliar spray resulted in the highest values for Sakha 95 under normal irrigation in the second season.

Proline Content of Leaves ($\text{mg g}^{-1} \text{FW}$)

Data as in Fig. 8 displayed that the highest levels of proline were recorded by the foliar application of Cina green plus under most conditions, while the lowest values were obtained by control treatment under all conditions. In addition, spraying using Lithovit with boron showed the highest values under water stress conditions for Sakha 95 in the second season and Misr 1 in the first season.

Total Chlorophyll Content (mg L^{-1})

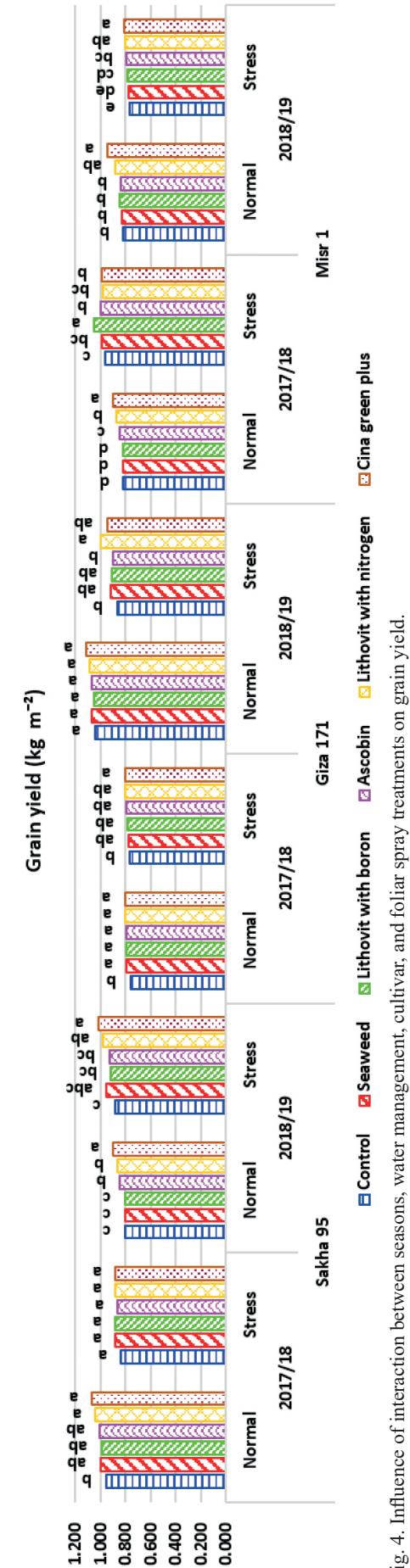
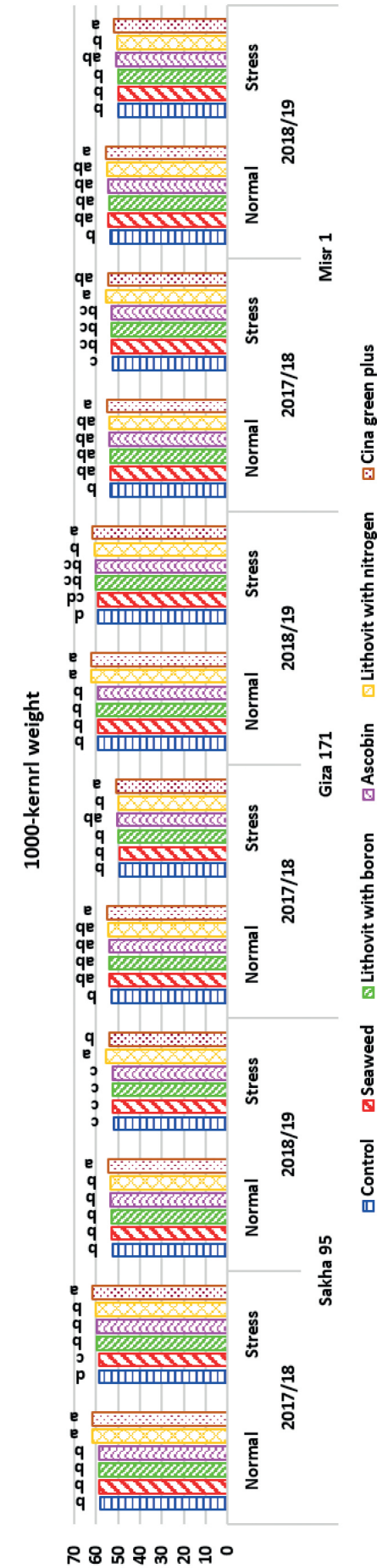
The foliar application of Cina green plus in Fig. 9 had the highest estimates of total chlorophyll content under most conditions compared to the control treatment. Moreover, spraying Lithovit with nitrogen had the highest estimates for Sakha 95 under normal conditions in the second season.

Grain Protein Content (%)

As illustrated in Fig. 10, spraying with Cina green plus generally resulted in the highest grain protein content across various conditions, surpassing the control treatment in all conditions. Additionally, Lithovit with boron application proved most effective in enhancing protein content for Sakha 95 consistently in the second season, for Giza 171 under normal irrigation in the first season, and for Misr 1 under normal irrigation in the first season. Furthermore, foliar application of Lithovit with nitrogen yielded the highest values for Misr 1 under normal irrigation in the second season. Notably, Seaweed application in the second season led to the highest protein content for Sakha 95 under normal irrigation and for Giza 171 under water stress.

Germination (%)

Our results in Fig. 11 show that foliar application of Cina green plus yielded the highest germination percentage for Giza 171 under water stress in the second season and Misr 1 under both water stress in the first season and normal irrigation in the second season. Additionally, Lithovit with boron application proved most effective for Sakha 95 under normal irrigation in the first season and water stress in the second season. Furthermore, spraying with Lithovit with nitrogen resulted in the highest germination rates for Giza 171 under normal irrigation in the second season, and Misr 1 under normal irrigation in the first season and water stress in the second season. Notably, Seaweed application yielded the highest germination percentage for Sakha 95 under normal irrigation in the second season, for Giza 171 under all irrigation treatments in the first season, and for Misr 1 under normal irrigation in the second season.



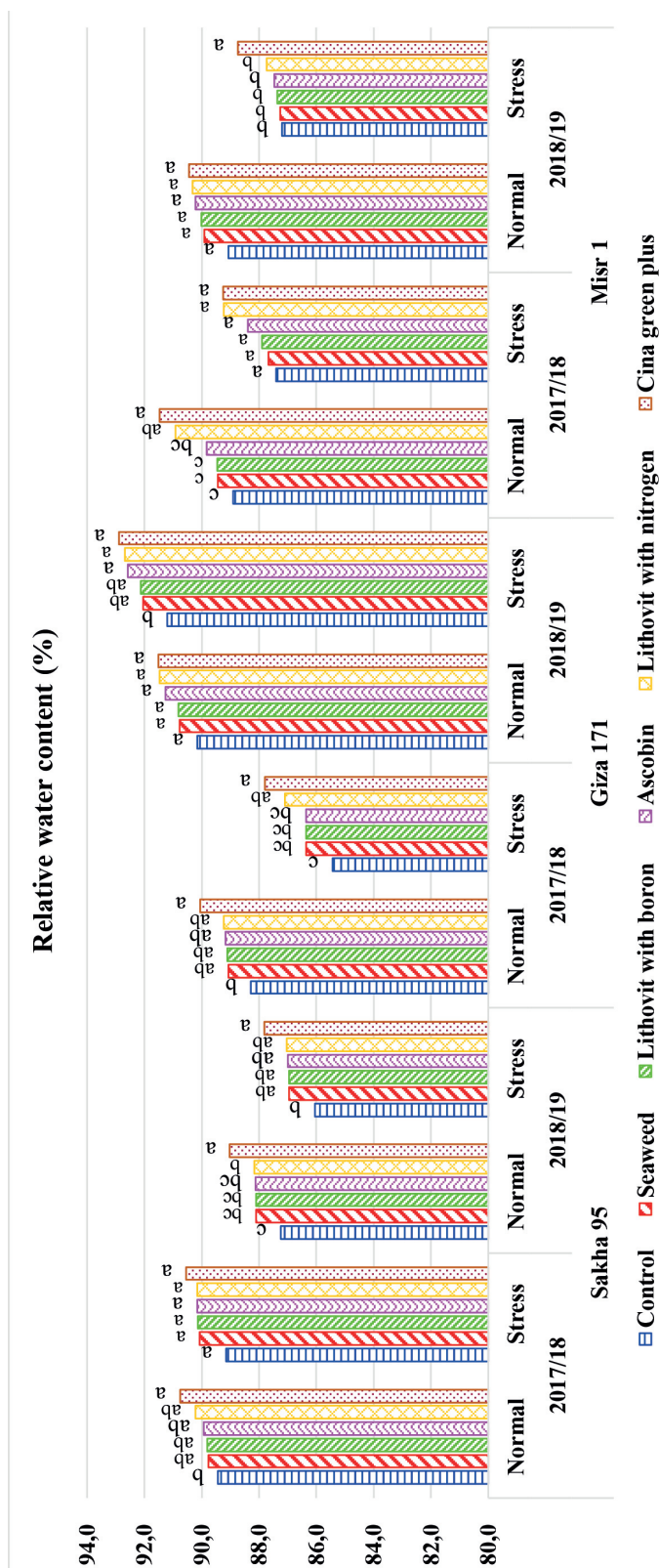


Fig. 5. Influence of interaction between seasons, water management, cultivar, and foliar spray treatments on relative water content.

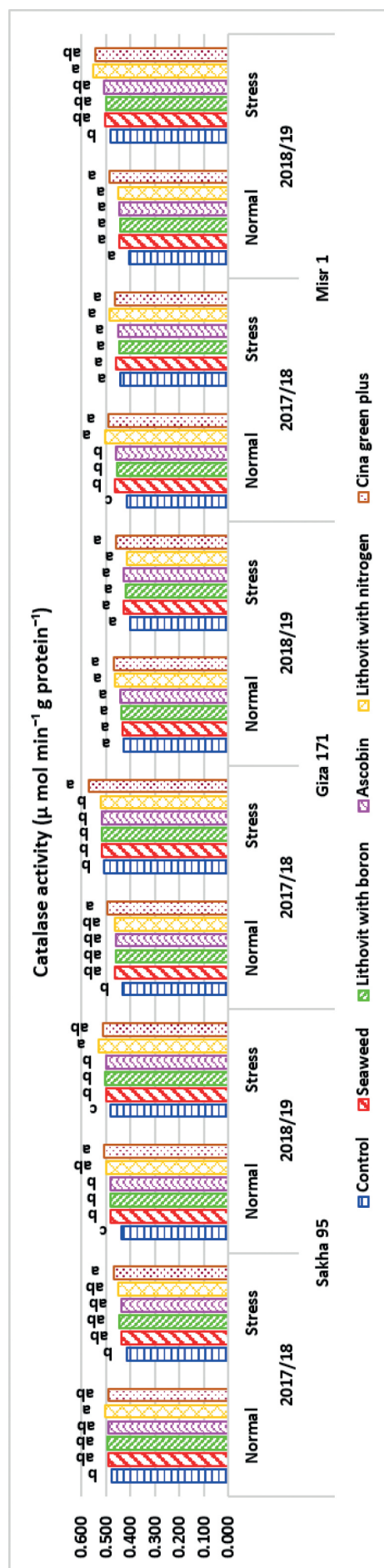


Fig. 6. Influence of interaction between seasons, water management, cultivar, and foliar spray treatments on catalase activity.

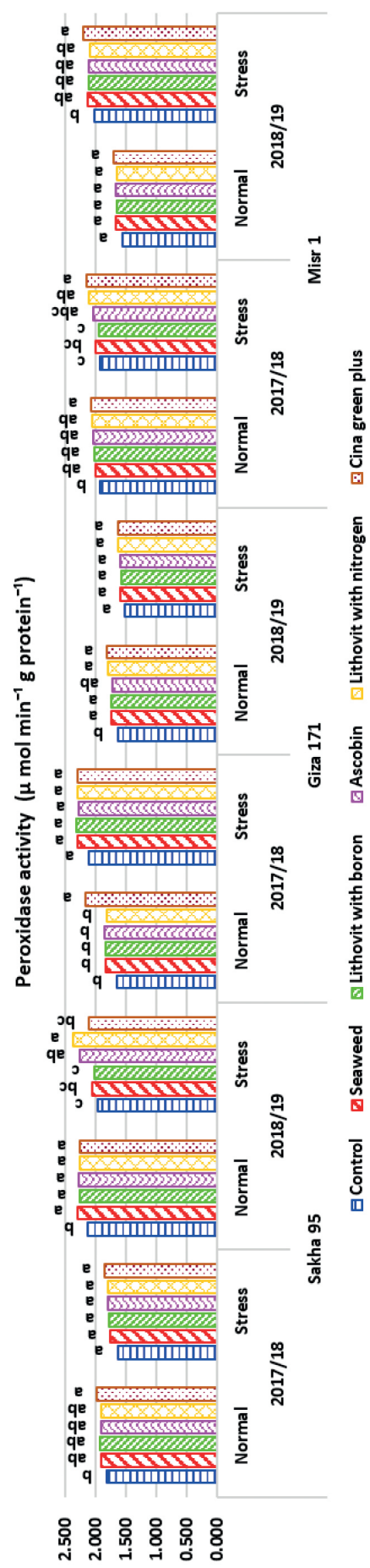


Fig. 7. Influence of interaction between seasons, water management, cultivar, and foliar spray treatments on peroxidase activity.

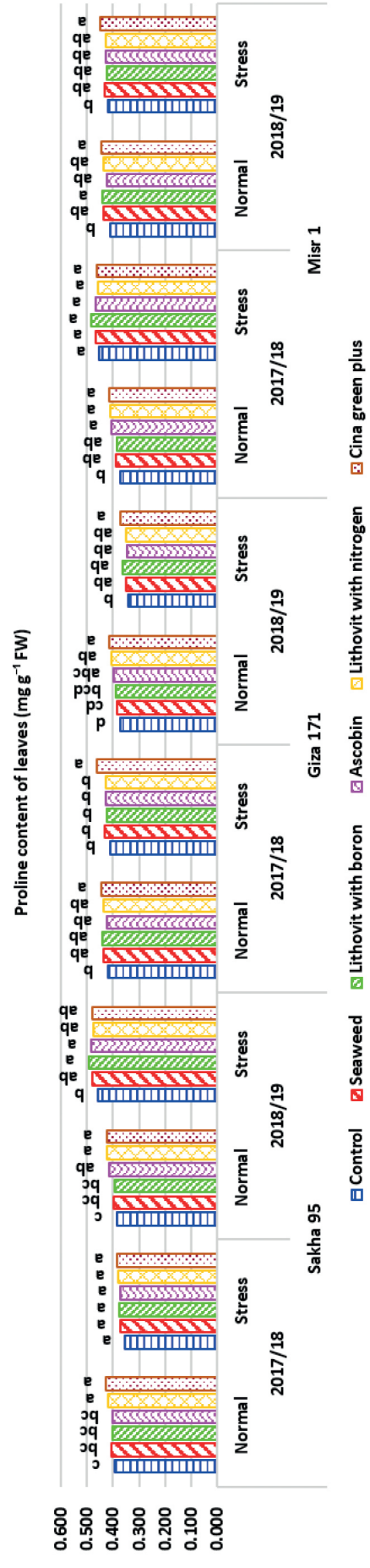


Fig. 8. Influence of interaction between seasons, water management, cultivar, and foliar spray treatments on proline content of leaves.

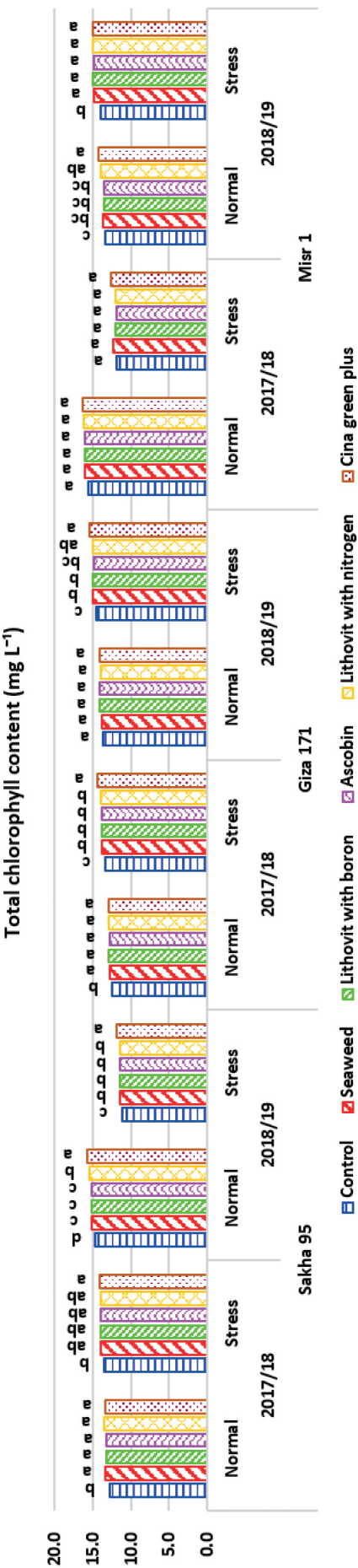


Fig. 9. Influence of interaction between seasons, water management, cultivar, and foliar spray treatments on total chlorophyll content.

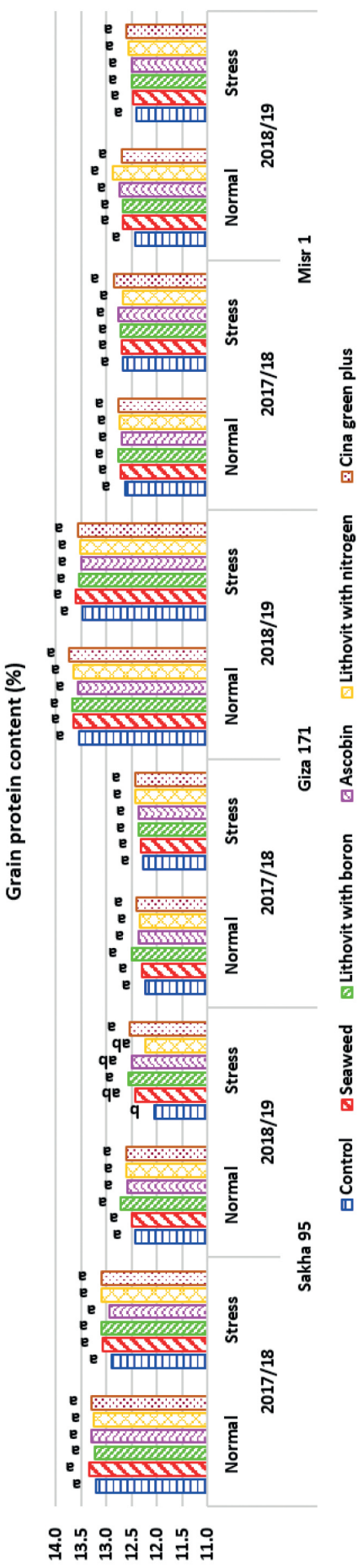


Fig. 10. Influence of interaction between seasons, water management, cultivar, and foliar spray treatments on grain protein content.

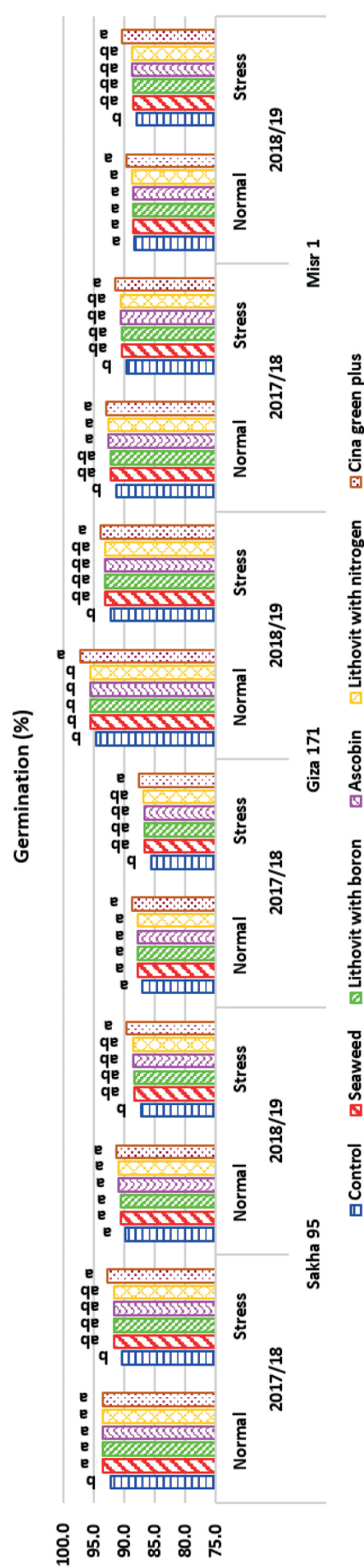


Fig. 11. Influence of interaction between seasons, water management, cultivar, and foliar spray treatments on germination %.

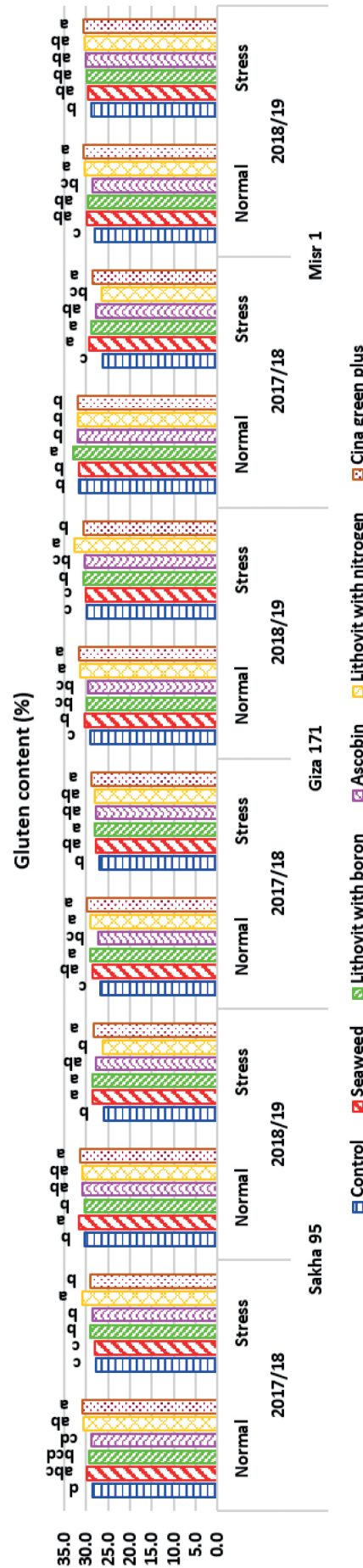


Fig. 12. Effect of the interaction between seasons, water treatments, cultivars, and foliar spray treatments on gluten content %.

Gluten Content (%)

According to Fig. 12, applying Cina green plus as a foliar spray consistently resulted in the highest gluten content across various conditions, while the control treatment consistently yielded the lowest. For the no. of spikes m^{-2} , Cina green plus again showed the most favorable results under most conditions compared to the control treatment. However, Lithovit with boron spray proved most effective for Misr 1 under normal irrigation in the first season. Additionally, spraying with Lithovit with nitrogen yielded the highest values for Sakha 95 under water stress in the first season and Giza 171 in the second season. Furthermore, applying Seaweed as a foliar spray resulted in the highest values for Sakha 95 under all irrigation treatments in the second season and for Misr 1 under water deficit stress in the first season.

Discussion

The error variances were proved to be homogeneous for the two seasons and irrigation treatments for all characters, so the combined analysis was performed across the two seasons and irrigation treatments. The study found statistically significant effects from seasons, water treatments, cultivars, and foliar sprays, indicating that these factors introduced a sufficient variation in the data [13]. The generally higher values observed for most studied traits in the 2018/19 season might be attributed to the low temperatures and high relative humidity compared to the 2017/18 season. Our results are consistent with previous results that reported a reduction in most wheat agronomic traits [31, 32]. When plants experience water stress, their cells face a build-up of osmotic pressure, which ultimately hinders their growth and ability to produce crops [33]. 1000-kernel weight, a key indicator of flour yield, is heavily influenced by post-flowering development stages and environmental factors [34]. Water stress decreases grain weight by shortening the grain filling period [35]. On the contrary, increasing in catalase and peroxidase activity, as well as proline content in wheat under water deficit stress, was confirmed. These results were confirmed by many researchers [36]. The increase in proline has been reported by many researchers during drought, and it helps plants stabilize membranes and cellular redox potential by scavenging ROS [37]. Increased proline can facilitate the maintenance of cellular osmotic potential under stress [38].

A plant's relative water content (RWC) acts as a reliable indicator of its ability to tolerate drought in many plants [39–41]. Studies show that drought-resistant varieties are capable of maintaining higher RWC even under limited water supply. Water stress negatively impacts a plant's water balance by increasing transpiration from stressed leaves while reducing its osmotic potential. This phenomenon has been extensively documented by many researchers [42].

Under drought conditions, chlorophyll content was decreased because of the effect of some special enzymes

on chlorophyll degradation [43]. Severe drought conditions can significantly hinder nitrogen uptake in plants by limiting the rate at which nitrogen is converted into usable forms in the soil and restricting the movement of ions. Additionally, water stress reduces the water potential gradient between seeds and their surrounding environment, leading to a decrease in germination percentage [44]. These findings align with previous research reporting varying responses of wheat cultivars to germination under diverse stress conditions [45]. Notably, the Masr 1 cultivar consistently exhibited the lowest final germination percentage under stress conditions [45].

Studies have shown that Lithovit can benefit plants in several ways. It has been linked to increased CO_2 levels, leading to improved photosynthesis [21]. Additionally, it contains essential nutrients that enhance chlorophyll and carotenoid production while also promoting better water movement within plants under saline conditions [20]. Research suggests similar positive effects with nano-calcium, which has been observed to improve plant growth and dry weight and mitigate the negative impacts of salinity [46, 47]. The application of Lithovit has also been linked to improvements in chlorophyll content, dry matter, and various growth parameters in tomatoes and wheat under salinity stress or natural conditions [22]. Furthermore, studies have demonstrated that the foliar application of Lithovit can enhance potato growth characteristics, tuber number, and overall yield per plant [48].

Catalase, a crucial enzyme, safeguards plant cells from oxidative damage caused by stress [49]. Foliar treatments significantly increased catalase activity compared to the control group, mitigating the harmful effects of ROS on the organelles under stress by converting destructive ROS into harmless compounds. This enzyme specifically converts H_2O_2 into water (H_2O) and oxygen (O_2). Previous research suggested that applying nano-nitrogen fertilizer can enhance various aspects of plant growth and yield [50]. This includes increases in total chlorophyll content, number of tillers per m^2 , number of spikelets per spike, 1000-grain weight, and both straw and grain yields. The study revealed that the Misr 1 cultivar achieved the highest levels of total chlorophyll, spike length, grain yield, and 1000-grain weight when treated with a combination of 120 kg of mineral nitrogen and 14 L of nano-nitrogen per hectare.

Conclusions

Based on the previous findings, it can be concluded that increasing nitrogen availability plays a crucial role in enhancing both nutrient efficiency and yield-related characteristics. Cina green plus foliar application and Lithovit with nitrogen could potentially enhance both the growth and yield of wheat crops, even under water stress conditions. These treatments hold promise as fertilizer options to mitigate the negative effects of water stress on wheat production.

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Data availability statement

All data that support the findings of this study are included in the article.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. AL-SHAMMARI WB., ALTAMIMI HR., ABDELAAL K. Improvement in Physiobiochemical and Yield Characteristics of Pea Plants with Nano Silica and Melatonin under Salinity Stress Conditions. *Horticulturae*. **9**, 711, **2023**.
2. ABDELAAL KH., MAZROU Y., HAFEZ Y. Silicon Foliar Application Mitigates Salt Stress in Sweet Pepper Plants by Enhancing Water Status, Photosynthesis, Antioxidant Enzyme Activity and Fruit Yield. *Plants*. **9**, 733, **2020**.
3. ABDELAAL K.H., ALSUBEIE M., HAFEZ Y., EMERAN A., MOGHANM F., OKASHA S., OMARA R., BASAHI M., DARWIS D., IBRAHIM M., ABOU EL-YAZIED A., RASHWAN E., ELKELISH A., MADY M., IBRAHEEM F. Physiological and Biochemical Changes in Vegetable and Field Crops under Drought, Salinity and Weeds Stresses: Control Strategies and Management. *Agriculture*. **12**, 2084, **2022**.
4. ABDELAAL KH., EL-OKKIAH S., METWALY M., EL-AFRY L. Impact of Ascorbic acid and proline application on the physiological machinery in soybean plants under salinity stress. *Fresenius Environmental Bulletin*. **30** (11A), 12486, **2021**.
5. EL SABAGH A., HOSSAIN A., BARUTCULAR C., ISLAM M., AWAN S.I., GALAL A., IQBAL A., SYTAR O., YILDIRIM M., MEENA R.S., FAHAD S., NAJEEB U., KONUSKAN O., HABIB R.A., LLANES A., HUSSAIN S., FAROOQ M., HASANUZZAMAN M., ABDELAAL KH., HAFEZ Y., CIG F., SANEOKA H. Wheat (*Triticum aestivum* L.) production under drought and heat stress-adverse effects, mechanisms and mitigation: A review. *Applied Ecology and Environmental Research*. **17** (4), 8307, **2019**.
6. ABDELAAL K.H., MAZROU Y., MOHAMED A., GHAZY M., BARAKAT M., HAFEZ Y., GABALLAH M. The different responses of rice genotypes to heat stress associated with morphological, chlorophyll and yield characteristics. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. **49** (4), 12550, **2021**.
7. ARAFA S.A., ATTIA K.A., NIEDEBALA G., PIEKUTOWSKA M., ALAMERY S., ABDELAAL K.H., ALATEEQ T.K., ALI M., ELKELISH A., ATTALLAH S.H.Y. Seed Priming Boost Adaptation in Pea Plants under Drought Stress. *Plants*. **10**, 2201, **2021**.
8. KHAFFAGY A.E., MAZROU Y.S.A., MORSY A.R., EL-MANSOURY M., EL-TOKHY A.I., HAFEZ Y., ABDELAAL K., KHEDR R.A. Impact of Irrigation Levels and Weed Control Treatments on Annual Weeds, Physiological Traits and Productivity of Soybean under Clay Soil Conditions. *Agronomy*. **12** (5), 1037, **2022**.
9. ALKAHTANI M.D.F., HAFEZ Y., ATTIA K., RASHWAN E., HUSNAIN L.A., ALGWAIZ H., ABDELAAL K.H. Evaluation of Silicon and Proline Application on the Oxidative Machinery in Drought-Stressed Sugar Beet. *Antioxidants*. **10** (3), 398, **2021**.
10. RASHWAN E., ALSOHIM A.S., EL-GAMMAAL A., HAFEZ Y., ABDELAAL K.H. Foliar application of nano zink-oxide can alleviate the harmful effects of water deficit on some flax cultivars under drought conditions. *Fresenius Environmental Bulletin*. **29** (10), 8889, **2020**.
11. ALSHAMMARI W., ALSHAMMERY K., LOTFI S., ALTAMIMI H., ALSHAMMARI A., AL-HARBI N., JAKOVLJEVIĆ D., ALHARBI M., MOUSTAPHA M., ABD EL-MONEIM D., ABDELAAL K.H. Improvement of morphophysiological and anatomical attributes of plants under abiotic stress conditions using plant growth-promoting bacteria and safety treatments. *PeerJ*. **12**, e17286, **2024**.
12. WASAYA A., MANZOOR S., YASIR T.A., SARWAR N., MUBEEN K., ISMAIL I.A., RAZA A., REHMAN A., HOSSAIN A., EL SABAGH A. Evaluation of Fourteen Bread Wheat (*Triticum aestivum* L.) Genotypes by Observing Gas Exchange Parameters, Relative Water and Chlorophyll Content, and Yield Attributes under Drought Stress. *Sustainability*. **13**, 4799, **2024**.
13. SHALABY E., GALALL E., ALI M., AMRO A., EL RAMLY A. Growth and yield responses of ten wheat (*Triticum aestivum* L.) genotypes to drought. *SVU-International Journal of Agricultural Sciences*. **2** (2), 1, **2020**.
14. KHADR S.A., EL-HAMAMSY S.M., EL-KHAMISSI H.A., SAAD Z.H. The effect of ascorbic acid treatment on wheat (*Triticum aestivum* L.) seedlings under drought stress. *Egyptian Journal of Applied Science*. **36** (1), 30, **2021**.
15. CALVO P., NELSON L., KLOEPFER J.W. Agricultural uses of plant biostimulants. *Plant Soil*. **383**, 3, **2019**.
16. STIRK W.A., VAN STADEN J. Plant growth regulators in seaweeds: Occurrence regulation and functions. *Advances in Botanical Research*. **71**, 125, **2014**.
17. KUREPIN L.V., ZAMAN M., PHARIS R.P. Phytohormonal basis for the plant growth promoting action of naturally occurring biostimulators. *Journal of the Science of Food and Agriculture*. **94**, 1715, **2014**.
18. ABDELAAL K.H., EL-MAGHRABY L.M., ELANSARY H., HAFEZ Y.M., IBRAHIM E.I., EL-BANNA M., EL-ESAWI M., ELKELISH A. Treatment of Sweet Pepper with Stress Tolerance-Inducing Compounds Alleviates Salinity Stress Oxidative Damage by Mediating

- the Physio-Biochemical Activities and Antioxidant Systems. *Agronomy*. **10**, 26, **2020**.
19. PAPENFUS H.B., KULKARNI M.G., STIRK W.A., FINNIE J.F., VAN STADEN J. Effect of a commercial seaweed extract (Kelpak®) and polyamines on nutrient-deprived (N, P and K) okra seedlings. *Scientia Horticulturae*. **151**, 142, **2013**.
 20. ISSA D., ALTURKI S., SAJYAN T., SASSINE Y. Sorbitol and lithovit-guano25 mitigates the adverse effects of salinity on eggplant grown in pot experiment. *Agronomy Research*. **18**, 113, **2020**.
 21. BILAL B. Lithovit: an innovative fertilizer. Presented at the 3rd e-Conference on Agricultural BioSciences (IeCAB 2010). Available online: <http://www.m.elewa.org/econferenceIeCAB.php>. **2010**.
 22. SAJYAN T.K., SHABAN N., RIZKALLAH J., SASSINE Y.N. Performance of salt- stressed tomato crop as affected by nano-caco 3, glycine betaine, MKP fertilizer and aspirin application. *Poljoprivreda i Sumarstvo*. **65**, 19, **2019**.
 23. ABDULLAH M., ABOU EI-YAZIED A., EL-MOGY M.M., ABDELDAYM E.A., ABDELAZIZ S.M., ABDELAAL K.H., IBRAHIM H. Extending the Shelf-Life of Lettuce Heads by Dipping in Phytic acid, cysteine, Methionine, and Ascorbic acid during Cold Storage. *Fresenius Environmental Bulletin*. **32** (3), 1458, **2023**.
 24. GONZALEZ L., GONZALEZ-VILAR M. Determination of relative water content. In: Reigosa, M. J. (Ed.), *Handbook of Plant Ecophysiology Techniques*. Kluwer Academic Publishers, Dordrecht 207, **2001**.
 25. BATES L.S., WALDEN R.P., TEARE I.D. Rapid determination of free proline for water studies. *Plant Soil*. **39**, 205, **1973**.
 26. LUM M.S., HANAFI M., RAFII Y.M., AKMAR A.S.N. Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. *Journal of Animal and Plant Sciences*. **24** (5), 1487, **2014**.
 27. AOAC. Official Methods of Analysis of the Association of Official Chemists, 15th ed., published by Association of Official Analytical Chemists Arlington, Virginia, U.S.A **1990**.
 28. AACC. Approved methods of the American association of cereal chemists, St. Paul, MN, USA, **1983**.
 29. LEVENE H. Robust test for equality of variances. In *Contributions to Probability and Statistics*. I. Olkin Ed., pp. 278–292, Stanford University Press, California. **1960**.
 30. PAYNE W., MURRAY D.A., HARDING S.A. An introduction to the GenStat command language. Hemel Hempstead, VSN International, UK. **2017**.
 31. FARHAT W.Z.E., KHEDR R.A., SHAABAN S.A. Response of some agronomic, physiological and anatomical characters for some bread wheat genotypes under water deficit in north delta region. *Scientific Journal of Agricultural Sciences*. **3** (2), 145, **2021**.
 32. OZTURK A., ERDEM E., AYDIN M., KARAOGLU M.M. The effects of drought after anthesis on the grain quality of bread wheat depend on drought severity and drought resistance of the variety. *Cereal Research Communications*. **50** (1), 105, **2021**.
 33. ZHANG H., ZHU J., GONG Z., ZHU J.K. Abiotic stress responses in plants. *Nature Reviews Genetics*. **23**, 104, **2021**.
 34. THALMANN M., SANTELIA D. Starch as a determinant of plant fitness under abiotic stress. *New Phytology*. **214**, 943, **2017**.
 35. SATTAR A., SHER A., IJAZ M., UL-ALLAH S., RIZWAN M.S., HUSSAIN M., JABRAN K., CHEEMA M.A. Terminal drought and heat stress alter physiological and biochemical attributes in flag leaf of bread wheat. *PLoS One*. **15** (5), **2020**, e0232974.
 36. LOUTFY N., EL-TAYEB M.A., HASSANEN A.M., MOUSTAFA M.F., SAKUMA Y., INOUE M. Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (*Triticum aestivum* L.). *Journal of Plant Research*. **125**, 173, **2012**.
 37. HUSSAIN M., FAROOQ S., HASAN W., UL-ALLAH S., TANVEER M., FAROOQ M. Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agricultural Water Management*. **201**, 152, **2018**.
 38. SUN C., PALMQVIST S., OLSSON H., BORE'N M., JANSSON A.C. A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the isol promoter. *Plant Cell*. **15**, 2076, **2003**.
 39. MARČEK M., ŠIMKOVÁ H., ŠTORCHOVÁ H. Relative water content as a measure of drought stress in plants: A review. *Agronomy*. **9** (4), 119, **2019**.
 40. ABDELAAL K.H. Effect of Salicylic acid and Absciscic acid on morpho-physiological and anatomical characters of faba bean plants (*Vicia faba* L.) under drought stress. *Journal of Plant Production*. **6** (11), 1771, **2015**.
 41. EL SABAGH A., HOSSAIN A., BARUTÇULAR C., ABDELAAL K.H., FAHAD S., ANJORIN F.B., ISLAM M.S., RATNASEKERA D., KIZILGEÇİ F., YADAV S., YILDIRIM M., KONUSKAN O., SANEOKA H. Sustainable maize (*Zea mays* L.) production under drought stress by understanding its adverse effect, Survival mechanism and drought tolerance indices. *Journal of Experimental Biology and Agricultural Sciences*. **6** (2), 282, **2018**.
 42. FAROOQ M., HUSSAIN M., UL-ALLAH S., SIDDIQUE K.H. Physiological and agronomic approaches for improving water-use efficiency in crop plants. *Agricultural Water Management*. **219**, 95, **2019**.
 43. KHEDR R., ABOUKHADRAH S., EL- HAG D., ELMOHAMADY E., ABDELAAL K.H. Ameliorative effects of nano silica and some growth stimulants on water relations, biochemical and productivity of wheat under saline soil conditions. *Fresenius Environmental Bulletin*. **32** (1), 375, **2023**.
 44. ALMAGHRABI O.A. Impact of drought stress on germination and seedling growth parameters of some wheat cultivars. *Life Science Journal*. **9** (1), 590, **2011**.
 45. METWALI M.R., EID M.H., BAYOUMI Y. Agronomical traits and biochemical genetic markers associated with salt tolerance in wheat cultivars (*Triticum aestivum* L.). *Australian Journal of Basic and Applied Science*. **5** (5), 174, **2011**.
 46. EL-FLAAH R.F., EL-SAID R.A.R., NASSAR M.A., HASSAN M., ABDELAAL K.H. Effect of rhizobium, nano silica and ascorbic acid on morpho-physiological characters and gene expression of POX and PPO in faba bean (*Vicia faba* L.) Under salinity stress conditions. *Fresenius Environmental Bulletin*. **30** (6) 5751, **2021**.
 47. ABD-EL-ATY M.S., KAMARA M.M., ELGAMAL W.H., MESBAH M.I., ABOMARZOKA E., ALWUTAYD K.M., MANSOUR E., ABDELMALEK I., BEHIRY S., ALMOSHADAK A.S., ABDELAAL K.H. Exogenous application of nano-silicon, potassium sulfate, or proline enhances physiological parameters, antioxidant enzyme activities, and agronomic traits of diverse rice

- genotypes under water deficit conditions. *Heliyon*. **10** (5), e26077, **2024**.
48. FAROUK S. Improving growth and productivity of potato (*Solanum tuberosum* L.) by some biostimulants and lithovit with or without boron. *Journal of Plant Production*. **6**, 2187, **2015**.
49. ZEESHAN M., LU M., SEHAR S.H., HOLFORD P., WU F. Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. *Agronomy*. **10**, 127, **2020**.
50. SAAD A.M., ALABDALI A.Y.M., EBAID M., SALAMA E., EL-SAADONY M.T., SELIM S., SAFHI F.A., SM A.L., ABDALLA H., MAHDI A.H.A., EL-SAADONY F.M.A. Impact of green chitosan nanoparticles fabricated from shrimp processing waste as a source of nano nitrogen fertilizers on the yield quantity and quality of wheat (*Triticum aestivum* l.) cultivars. *Molecules*. **27** (17), **2022**.