



Enhancing Drought Resistance in Wheat (*Triticum aestivum* L.) Seedlings by Aqueous Extract of *Spirulina platensis*

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Abstract: Drought is a significant constraint for growth and development, especially at the juvenile stage of wheat, a vital global crop. This study investigated the effects of drought and the potential of grain presoaking in *Spirulina platensis* aqueous extract (SPAЕ) on two distinct wheat cultivars (Shandawel 1 and Sakha 95). Parameters assessed included plumule and radicle length, the number of adventitious roots, fresh and dry mass, seedling water content, and germination percentage. Drought led to a decrease in most growth parameters but increased seedling dry mass. In comparison to presoaking in distilled water, presoaking in SPAЕ significantly mitigated the adverse effects of drought in both cultivars. Specifically, *Spirulina platensis* treatments enhanced plumule and radicle length, seedling fresh mass, and germination percentage in stressed seedlings. The study demonstrates the promising role of SPAЕ as a potential stress-alleviating agent in wheat cultivation under drought conditions, with varying effects observed across different wheat cultivars. The findings shed light on innovative strategies to improve wheat growth and development in drought-prone regions.

Key words: Wheat cultivars, germination stage, *Spirulina platensis*, grain presoaking, drought resistance.

Introduction

Wheat (*Triticum aestivum* L.) is the most widely cultivated crop and is critical to global food security [1]. One-third of the world's population relies on wheat as a staple food, making it the "king of cereals" [2]. Wheat is highly valued for its nutritional properties, serving as a rich source of carbohydrates, proteins, vitamins, and minerals [3]. In addition, wheat grains contain phytochemicals that provide health benefits and complement those found in vegetables and fruits [4]. In Egypt, wheat is the foremost strategic food crop, being the most basic staple for breadmaking and sometimes mixed with maize. Furthermore, wheat straw is an important livestock fodder in many regions of Egypt [5].

However, drought is a major abiotic stress that limits the yield of major crops, especially wheat. Water deficits dramatically affect plant metabolism, resulting in cell membrane damage, organelle disruption, and impaired

physiological activities [6]. Germination and early seedling establishment are critical phases in the wheat life cycle that are highly susceptible to drought stress [7]. Water deficiency during germination leads to poor germination rates, delayed emergence, and abnormal seedling growth [8]. This has severe impacts on stand establishment, tillering, spike formation, and ultimately grain yield [9].

Developing techniques to improve wheat quality under drought stress is therefore imperative. Various exogenous stimulants have shown potential for enhancing drought resistance in plants. Currently, plant biostimulants derived from marine algae are attracting attention as promising solutions. Algal extracts contain diverse bioactive components like polysaccharides, phenols, mannitol, betaine, and phytohormones that can stimulate plant growth and stress tolerance [10]. For instance, *Gracilaria dura* extract increased

wheat yield under drought by 70% by stimulating abscisic acid signaling [10]. Other algal extracts also improve drought tolerance by promoting growth, photosynthesis, and related gene expression [11, 12].

However, further studies are warranted on the effects of algal biostimulants specifically during the germination stage. Optimizing germination and early seedling vigor is key to ensuring uniform stand establishment and high productivity under drought stress [13, 14]. This highlights the need to investigate algal extracts as seed treatments to improve the drought resilience of wheat during this critical phase.

Plant Materials

Triticum aestivum (wheat) genotypes

Pure strains of two wheat genotypes; most tolerant (Sakha 95) and most sensitive ones (Shandawel 1)" were obtained from the Egyptian agricultural Research Center, Sakha, Egypt.

Spirulina platensis alga

Cultivation of *Spirulina platensis*; Axenic *Spirulina platensis* culture was cultivated in sterilized Zarrouk's liquid medium [15], and 500 ml inoculum (10%) was inoculated with 5000 ml Zarrouk's medium in a 10 liters flask. The flask was incubated at 26 ± 2 under continuous illumination (cool- white fluorescent, 3600 lux, 14 days). Then, five liters inoculum was inoculated into 50 liters Zarrouk's medium in plastic growth tower and incubated under growth conditions.

Filtration and drying; *Spirulina* culture was filtered using special filters (nylon mesh filter). Then, the collected *spirulina* was dried using a freeze dryer "lypholizer" for 48h at -50°C .

Preparation of *Spirulina platensis* aqueous extract; *Spirulina platensis* extract was prepared by homogenising 5 g of *Spirulina platensis* alga with 50 ml of distilled water and then filtering using a suction pump. The filtrate is then collected and used as an algal extract.

Evaluation of Spirulina platensis

Estimation of dry weight biomass

At the end of incubation, the culture of *Spirulina* was harvested by centrifugation at 400rpm for 10 minutes., then washed with distilled water to remove any salt remains. The

pellet of the *Spirulina* was dried in a freeze dryer for 48h at -50°C and weighed [16].

Photosynthetic activity using PAM fluorometer (daily)

Photosynthetic activity was measured every day using a PAM fluorometer (AquaPen 101 C) to determine the stress of the culture based on the values of Fv/Fm.

Chlorophyll a, b and Carotenoids Determination

The pigment fractions (chlorophyll a, b and carotenoids) were determined by the spectrophotometric method [17].

Estimation of Protein

Protein content was determined according to Lowry's method [18]. The protein estimation was carried out by cell lysis and bovine serum albumin (BSA) as a standard.

Total Carbohydrate Determination

The methodology adopted in this study is derived from the phenol-sulphuric acid assay delineated in [19]. This assay enables the quantification of a comprehensive spectrum of carbohydrates, encompassing both reducing and non-reducing sugars, as well as polysaccharides, obviating the need for initial hydrolysis. The assay's sensitivity lies in its ability to detect the total monosaccharide units, making it independent of the carbohydrate's polymerisation state or configuration. Upon reaction, an orange hue emerges due to the interaction between carbohydrates and phenol, the absorbance of which is subsequently quantified at a wavelength of 490 nm and benchmarked against glucose calibration standards.

Total Lipid Determination

Lipid concentration estimated by sulfo-phospho-vanillin method described by [20], and using cholesterol as a standard.

Germination Experiment

Surface sterilization of a homogeneous lot of grains from the examined genotypes was achieved by soaking them for 3 minutes in a 2.5 percent sodium hypochlorite solution, followed by multiple washes with distilled water. The sterilized grains were presoaked for 12 hours in distilled water (first set) and *Spirulina platensis* aqueous extract (second set). The grains were

then allowed to drain for an hour.

Germination Experiment

A germination trial was carried out to investigate if algal extracts might be used to induce drought resistance in wheat seedlings. The presoaked grains in each set were then allowed to germinate in the dark at room temperature for 7 days in two groups: one was given water when needed as a control, while the other was given PEG 6000 at 15% (-2.95 bar osmotic pressure) as a treatment. The treatments of each cultivar are, control (**cont.**), drought stress (**DS**), *Spirulina platensis* aqueous extract (**SPAE-Cont**), and *Spirulina platensis* extract with drought stress (**SPAE + DS**).

Wheat seedlings were sampled at the end of the germination period to determine the effects of drought on the plants, and to explore the role of algal extracts in drought escape of the stressed wheat seedling and the effect of algal extracts on wheat seedling development characteristics. For that, the germination parameters including plumule and radicle length, the number of adventitious roots as well as the fresh and dry mass of 7-day old wheat seedlings were assessed. Seedling water content, the amount of water per unit seedling fresh mass, was additionally calculated as cited from [21]. The amount of water per unit seedling fresh mass, was calculated; Water content = fresh mass – dry mass / fresh mass.

Statistical analysis

For the germination parameters assay, five replicates were taken. Only the mean values were tabulated. Statistical analysis was conducted using CoHort/CoStat software. A one-way completely randomized ANOVA test with a significance level of $P \leq 0.05$ was employed. Replicates underwent descriptive analysis to determine standard deviation. Lowercase letters were subsequently generated. These letters serve as indicators of variation: different letters signify significant differences, and the greater the distance between the letters, the higher the degree of variation.

The percent of change in each estimated criterion for each treatment of each cultivar was recorded from 1WCR analysis as $100 \times [(drought\ value - control\ value) / control\ value]$. Corresponding figures were then illustrated as a

relation between the treatment of each cultivar on x- axis and the percent of change on y- axis for each parameter. The treatments of cultivar Shandawel 1 are, shandawel 1 presoaked in distilled water (**S1 + dist. H₂O**), and shandawel 1 presoaked in *Spirulina platensis* aqueous extract (**S1 + SPAE**). However, the treatments of cultivar Sakha 95 are Sakha 95 presoaked in distilled water (**S95 + dist. H₂O**), and Sakha 95 presoaked in *Spirulina platensis* aqueous extract (**S95 + SPAE**)

Results and discussion

Biochemical constituent of *Spirulina platensis*

The evaluation of *Spirulina platensis* reveals some significant findings related to its photosynthetic activity, composition, and nutritional content. In case photosynthetic activity, the observed mean value was 0.54 ± 0.04 . Regarding, the dry weight was measured at 0.43 ± 0.03 g/l.

The concentrations of chlorophyll-a (Chl a) and chlorophyll-b (Chl b) were 11.831 ± 0.8 mg/g and 10.036 ± 0.8 mg/g, respectively. These values represent the photosynthetic pigments in the algae. For Carotenoids (mg/g), the concentration was 2.334 ± 0.3 mg/g, providing insights into the presence of these antioxidative compounds. The protein content was notably high, at $54.9 \pm 10.0\%$. In contrast, the carbohydrate and lipid percentages were $7.5 \pm 0.2\%$ and $7.30 \pm 0.37\%$, respectively. The results contribute to our understanding of *Spirulina platensis*, a microalga known for its agricultural applications [22].

The consistent photosynthetic activity (0.54 ± 0.04) indicates a stable growth environment and energy conversion rate, reflecting the efficiency of the photosynthesis process in the examined *Spirulina platensis* samples [23].

A considerable amount of dry weight (0.43 ± 0.03 g/l) suggests the potential for industrial scaling, as this measurement is a key factor in assessing the economic feasibility of commercial production [24].

The higher levels of chlorophyll (Chl a 11.831 ± 0.8 mg/g and Chl b 10.036 ± 0.8 mg/g) and carotenoids (2.334 ± 0.3 mg/g) illustrate the presence of compounds known for their antioxidant properties, offering potential

applications in biofertilizer products [25].

The dominant protein content ($54.9 \pm 10.0\%$) signifies the promising use of *Spirulina platensis* as a protein source for plants [26]. The relatively low concentrations of carbohydrates and lipids underscore its suitability for plants.

In summary, the analysis of *Spirulina platensis* underlines its potential for various applications, ranging from plant biofertilizer and plant bio stimulant helping in stress alleviation driven by its substantial protein content, antioxidative compounds, and consistent photosynthetic activity.

SPAE alleviates drought-induced inhibition of wheat grain germination.

The pool of data obtained was statistically analyzed, tabulated in

Table (1) and

Table (2) and graphically represented in **Fig. (1)** and **Fig. (2)**. Regarding plumule length in Shandawel 1 and Sakha 95 wheat cultivars, notable variations were observed across different treatments. In the sensitive cultivar Shandawel 1, the SPAE-Cont treatment exhibited a plumule length of $14.3a \pm 0.3$ cm, significantly higher compared to the control value of $12.4b \pm 0.5$ cm. Conversely, drought stress (DS) reduced the plumule length to $5.3d \pm 0.4$ cm. When Shandawel 1 was subjected to a combination of drought stress and *Spirulina platensis* aqueous extract (DS+SPAE), the plumule length measured $11.3c \pm 0.4$ cm, indicating a mitigative effect on drought-induced reduction.

In the tolerant cultivar Sakha 95, the SPAE-Cont treatment yielded the longest plumules, with a length of $15.3a \pm 0.5$ cm, compared to $13.5b \pm 0.6$ cm in the control. Drought stress resulted in a plumule length of $10.4c \pm 0.1$ cm, while the DS+SPAE treatment recorded a length of $13.0b \pm 0.0$ cm, revealing a lesser but still notable alleviating effect compared to that in Shandawel 1.

The Least Significant Difference (LSD) at $P \leq 0.05$ for plumule length was 0.76 for Shandawel 1 and 0.74 for Sakha 95, underscoring the significance of these findings. In summary, the *Spirulina platensis* aqueous extract exhibits an alleviative effect on plumule

length in both sensitive and tolerant wheat cultivars under drought stress conditions.

In relation to radicle length in the wheat cultivars Shandawel 1 and Sakha 95, significant variations were evident among the different treatments. For the sensitive cultivar Shandawel 1, the SPAE-Cont treatment resulted in a radicle length of $18.2a \pm 1.0$ cm, which is markedly greater than the control value of $14.3b \pm 0.6$ cm. On the other hand, drought stress (DS) led to a significant reduction, with the radicle length dropping to $8.6c \pm 0.4$ cm. When subjected to both drought stress and *Spirulina platensis* aqueous extract (DS+SPAE), the radicle length measured $17.1a \pm 0.1$ cm, indicating an alleviative effect on drought-induced reductions.

In the tolerant cultivar Sakha 95, radicle length in the SPAE-Cont treatment was recorded as $19.5a \pm 1.4$ cm, exceeding the control length of $16.2b \pm 1.1$ cm. Drought stress (DS) resulted in a slightly reduced radicle length of $15.1b \pm 0.9$ cm, whereas the DS+SPAE treatment measured $18.4a \pm 0.4$ cm, suggesting a mitigating effect similar to that in Shandawel 1 but less pronounced.

The Least Significant Difference (LSD) at $P \leq 0.05$ for radicle length was 1.16 for Shandawel 1 and 1.88 for Sakha 95, further reinforcing the significance of the observed differences. To summarise, the *Spirulina platensis* aqueous extract appears to have a significant alleviative impact on radicle length in both sensitive and tolerant wheat cultivars under drought stress conditions.

Regarding the number of adventitious roots in Shandawel 1 and Sakha 95 wheat cultivars, distinct variations were evident among the treatments. For the sensitive cultivar Shandawel 1, the control (Cont.) showed an average of $4.7bc \pm 0.6$ adventitious roots. Under drought stress (DS), this number decreased to $4.0c \pm 0.0$. On the other hand, *Spirulina platensis* aqueous extract control (SPAE-Cont) treatment

significantly increased the number of adventitious roots to $5.7a \pm 0.6$, which was markedly higher than all other treatments. The number of adventitious roots in Shandawel 1

subjected to both drought stress and *Spirulina platensis* aqueous extract (DS+SPAE) was $5.3ab \pm 0.6$, indicating partial mitigation of

the drought-induction. In the tolerant cultivar Sakha

95, the control treatment yielded $5.0ab \pm 0.0$ adventitious roots. Drought stress led to a reduction in the number to $4.3b \pm 0.6$. Similarly, the SPAE-Cont treatment produced a higher number of adventitious roots, $5.7a \pm 0.6$, than the control. The DS+SPAE treatment resulted in a comparable number of adventitious roots, $5.3a \pm 0.6$, suggesting a mitigative effect against drought stress.

The Least Significant Difference (LSD) at $P \leq 0.05$ for the number of adventitious roots was 0.9 for Shandawel 1 and 0.94 for Sakha 95. These results underscore the significance of *Spirulina platensis* aqueous extract in positively influencing the number of adventitious roots in both sensitive and tolerant wheat cultivars when subjected to drought stress.

Regarding the percentage of germination in both Shandawel 1 and Sakha 95 wheat cultivars, distinct outcomes were observed among various treatments. In the sensitive cultivar Shandawel 1, the control and SPAE-Cont treatments both resulted in a maximum germination rate of $100a \pm 0\%$. Conversely,

drought stress (DS) led to a significant reduction in germination rate, registering $73b \pm 6\%$. Importantly, the combined drought stress and *Spirulina platensis* aqueous extract treatment (DS+SPAE) exhibited a germination rate of $93a \pm 6\%$, suggesting a mitigative effect on drought-induced germination reduction.

In the tolerant cultivar Sakha 95, both the control and SPAE-Cont treatments achieved full germination, with rates of $100a \pm 0\%$. The application of drought stress resulted in a slightly reduced germination rate of $90b \pm 0\%$. However, the DS+SPAE treatment revealed a near-maximum germination rate of $97a \pm 6\%$, affirming the extract's alleviative role in drought conditions.

The Least Significant Difference (LSD) at $P \leq 0.05$ for germination percentage was 8 for Shandawel 1 and 5 for Sakha 95, thereby establishing the statistical significance of these observations. In summary, the *Spirulina platensis* aqueous extract demonstrates a protective effect on the germination rates of both sensitive and tolerant wheat cultivars under drought stress conditions.

Table (1): Effect of grain presoaking in *Spirulina platensis* aqueous extract on germination features of droughted wheat cultivars. Data listed represents mean values \pm standard deviation. Different superscript letters refer to significant variation with the least significant difference (LSD) at $p \leq 0.05$. Low, moderate and high degree of significance is indicated by *, ** and *** while non-significant difference is abbreviated as ns.

Wheat cultivars	Parameters Treatments	Plumule length(cm)	Radicle length(cm)	Number of adventitious roots	% of germination
Sensitive (Shandawel 1)	Cont.	$12.4^b \pm 0.5$	$14.3^b \pm 0.6$	$4.7^{bc} \pm 0.6$	$100^a \pm 0$
	DS	$5.3^d \pm 0.4$	$8.6^c \pm 0.4$	$4.0^c \pm 0.0$	$73^b \pm 6$
	SPAE-Cont	$14.3^a \pm 0.3$	$18.2^a \pm 1.0$	$5.7^a \pm 0.6$	$100^a \pm 0$
	DS+ SPAE	$11.3^c \pm 0.4$	$17.1^a \pm 0.1$	$5.3^{ab} \pm 0.6$	$93^a \pm 6$
	LSD at $P \leq 0.05$	0.76	1.16	0.9	8
	Degree of Significance	***	***	*	***
Tolerant (Sakha 95)	Cont.	$13.5^b \pm 0.6$	$16.2^b \pm 1.1$	$5.0^{ab} \pm 0.0$	$100^a \pm 0$
	DS	$10.4^c \pm 0.1$	$15.1^b \pm 0.9$	$4.3^b \pm 0.6$	$90^b \pm 0$
	SPAE-Cont	$15.3^a \pm 0.5$	$19.5^a \pm 1.4$	$5.7^a \pm 0.6$	$100^a \pm 0$
	DS+ SPAE	$13.0^b \pm 0.0$	$18.4^a \pm 0.4$	$5.3^a \pm 0.6$	$97^a \pm 6$
	LSD at $P \leq 0.05$	0.74	1.88	0.94	5
	Degree of Significance	***	**	ns	**

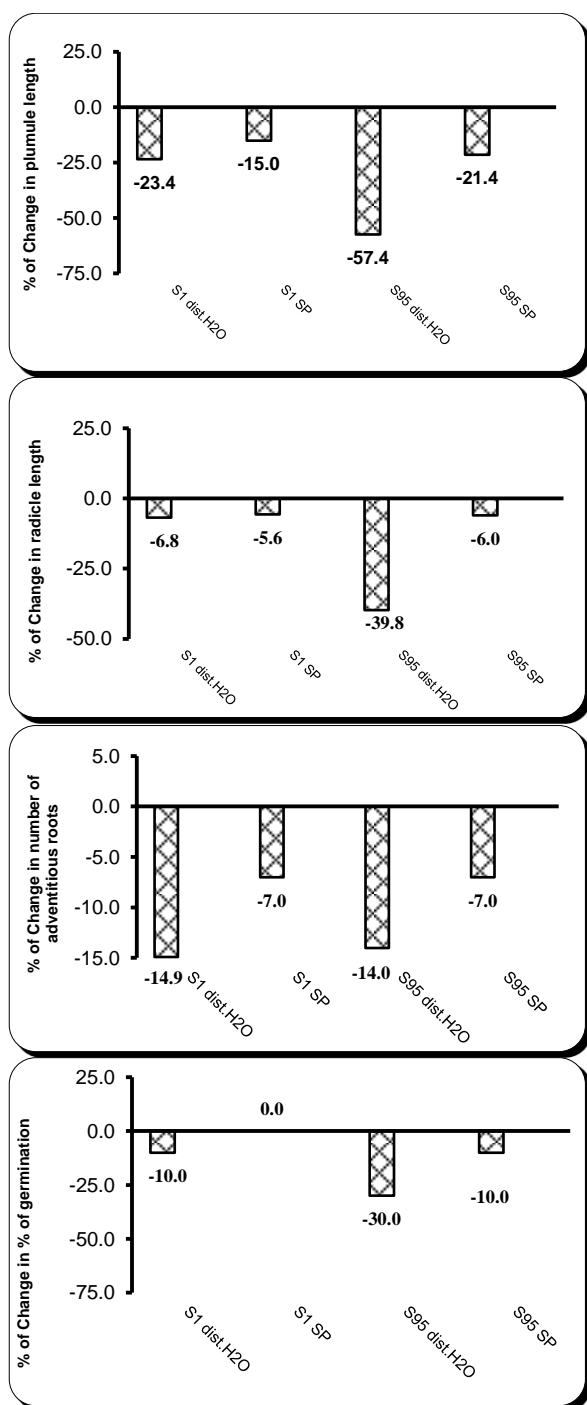


Fig. (1): Effect of grain presoaking in *Spirulina platensis* aqueous extract on % of change in plumule length, radicle length and number of adventitious roots of droughted wheat cultivars.

Concerning the seedling fresh mass in Shandawel 1 and Sakha 95 wheat cultivars, significant disparities were observed across different treatments. For Shandawel 1, the sensitive cultivar, the SPAE-Cont treatment resulted in the highest seedling fresh mass, measuring $235a \pm 7$ mg, substantially greater than the control group, which registered $172b \pm 20$ mg. On the contrary, drought stress (DS) led to a marked decline in seedling fresh mass,

recording $104c \pm 14$ mg. The combined treatment of drought stress and *Spirulina platensis* aqueous extract (DS+SPAE) yielded a seedling fresh mass of $167b \pm 2$ mg, indicating its mitigative role against drought-induced losses.

In the tolerant cultivar Sakha 95, the SPAE-Cont treatment yielded the greatest seedling fresh mass, amounting to $274.0a \pm 9.5$ mg, which is notably higher than the control mass of $226.3b \pm 17.2$ mg. Drought stress reduced the seedling fresh mass to $168.7c \pm 6.1$ mg, whereas the DS+SPAE treatment achieved a mass of $210.7b \pm 7.0$ mg, showing a lesser but still significant alleviative impact.

The Least Significant Difference (LSD) at $P \leq 0.05$ for seedling fresh mass was 24 for Shandawel 1 and 20 for Sakha 95, thereby confirming the statistical significance of the observed differences. In summary, the *Spirulina platensis* aqueous extract presents a beneficial effect on seedling fresh mass in both sensitive and tolerant wheat cultivars under drought conditions, showcasing its potential as a mitigative agent.

Regarding seedling dry mass in the sensitive wheat cultivar Shandawel 1, diverse responses to treatments were observed. The control group exhibited a dry mass of $30b \pm 3$ mg, which was substantially outperformed by the drought stress (DS) treatment, yielding $45a \pm 4$ mg. Intriguingly, despite a decline in fresh mass, drought stress led to a considerable increase in dry mass. For the *Spirulina platensis* aqueous extract control treatment (SPAE-Cont), the dry mass was measured at $35b \pm 3$ mg, whereas the combined treatment of drought stress and *Spirulina platensis* aqueous extract (DS+SPAE) further elevated the dry mass to $48a \pm 4$ mg.

For Sakha 95, the tolerant cultivar, the DS+SPAE treatment exhibited the highest dry mass at $49a \pm 3$ mg, outperforming the SPAE-Cont treatment, which recorded $46ab \pm 2$ mg. The control and drought stress treatments resulted in dry masses of $38c \pm 2$ mg and $42bc \pm 2$ mg, respectively.

In both cultivars, the DS+SPAE treatment was observed to enhance dry mass notably, underlining the mitigative potential of *Spirulina platensis* in drought conditions. The Least Significant Difference (LSD) at $P \leq 0.05$ for

seedling dry mass was 6 for Shandawel 1 and 4 for Sakha 95, confirming the statistical significance of these observations.

In summary, the application of *Spirulina platensis* aqueous extract, particularly when combined with drought stress, has been shown to significantly improve seedling dry mass in both sensitive and tolerant wheat cultivars, suggesting its utility in ameliorating the negative effects of environmental stressors on crop performance.

In terms of seedling water content, divergent patterns were observed across different treatments for both sensitive and tolerant wheat cultivars. For the sensitive cultivar Shandawel 1, the control treatment registered a seedling water content of $0.83a \pm 0.01$ mg H₂O mg⁻¹ fresh weight. The drought stress (DS) treatment resulted in a significant reduction to $0.57c \pm 0.02$. Interestingly, the *Spirulina platensis* aqueous extract control (SPAE-Cont) maintained a comparable water content of $0.85a \pm 0.01$, similar to the control. The combination

of drought stress and *Spirulina platensis* (DS+SPAE) recorded a water content of $0.72b \pm 0.02$, indicating an ameliorative effect on drought stress.

For the tolerant cultivar Sakha 95, the water content for the SPAE-Cont treatment was $0.83a \pm 0.01$, equalling that of the control treatment ($0.83a \pm 0.01$). Drought stress alone yielded a slightly decreased water content of $0.76b \pm 0.02$. The DS+SPAE treatment showed a marginal improvement to $0.77b \pm 0.02$ compared to the DS treatment.

The Least Significant Difference (LSD) at $P \leq 0.05$ was 0.03 for both cultivars, confirming the statistical significance of the observed variations in seedling water content. These findings suggest that *Spirulina platensis* aqueous extract, particularly in combination with drought stress, serves to mitigate the decrease in water content, thus reinforcing its potential utility for improving crop resilience under adverse environmental conditions.

Table (2): Effect of grain presoaking in *Spirulina platensis* aqueous extract on seedling fresh mass, dry mass and water content of droughted wheat cultivars. Data listed represents mean values \pm standard deviation. Different superscript letters refer to significant variation with the least significant difference (LSD) at $p \leq 0.05$. Low, moderate and high degree of significance is indicated by *, ** and *** while non-significant difference is abbreviated as ns.

Wheat cultivars	Parameters Treatments	Seedling fresh mass(mg)	Seedling dry mass(mg)	Seedling Water Content (mg H ₂ O mg ⁻¹ freshweight)
Sensitive (Shandawel 1)	Cont.	$172^b \pm 20$	$30^b \pm 3$	$0.83^a \pm 0.01$
	DS	$104^c \pm 14$	$45^a \pm 4$	$0.57^c \pm 0.02$
	SPAE-Cont	$235^a \pm 7$	$35^b \pm 3$	$0.85^a \pm 0.01$
	DS+ SPAE	$167^b \pm 2$	$48^a \pm 4$	$0.72^b \pm 0.02$
	LSD at $P \leq 0.05$	24	6	0.03
	Degree of Significance	***	***	***
Tolerant (Sakha 95)	Cont.	$226.3^b \pm 17.2$	$38^c \pm 2$	$0.83^a \pm 0.01$
	DS	$168.7^c \pm 6.1$	$42^{bc} \pm 2$	$0.76^b \pm 0.02$
	SPAE-Cont	$274.0^a \pm 9.5$	$46^{ab} \pm 2$	$0.83^a \pm 0.01$
	DS+ SPAE	$210.7^b \pm 7.0$	$49^a \pm 3$	$0.77^b \pm 0.02$
	LSD at $P \leq 0.05$	20	4	0.03
	Degree of Significance	***	**	**

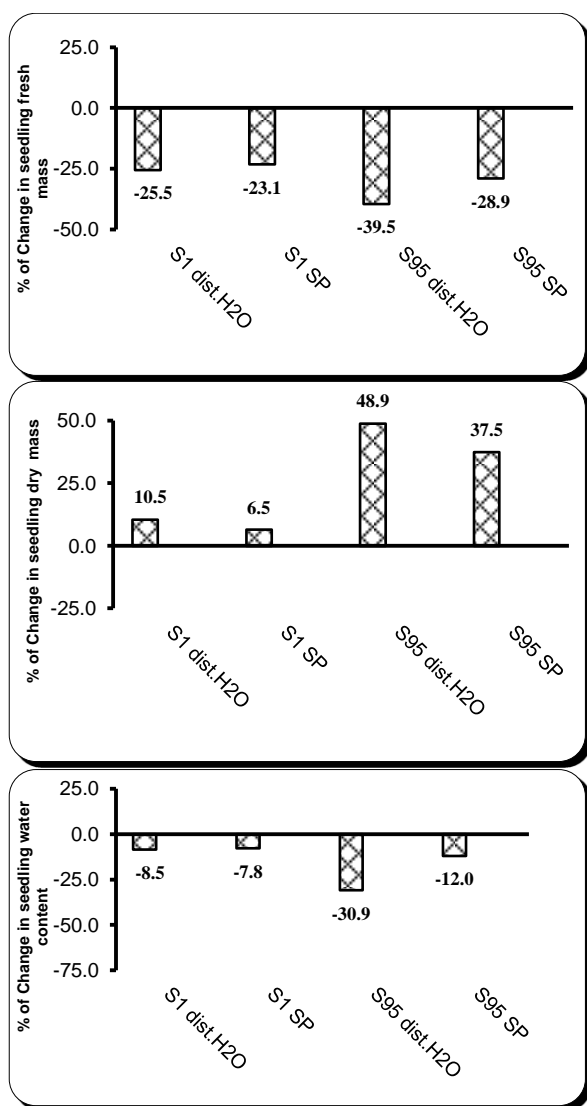


Fig. (2): Effect of grain presoaking in *Spirulina platensis* aqueous extract on % of change in seedling fresh mass, dry mass and water content of droughted wheat cultivars.

The results demonstrated that presoaking wheat grains in *Spirulina platensis* aqueous extract (SPAe) had an alleviative effect on several germination parameters under drought stress conditions. Overall, SPAe improved plumule length, radicle length, adventitious root number, germination percentage, and seedling fresh mass in both sensitive (Shandawel 1) and tolerant (Sakha 95) wheat cultivars subjected to drought.

These findings align with recent studies highlighting the positive impacts of algal extracts and other biostimulants on seed germination under abiotic stress. For instance, [27] showed that brown algal extract increased radicle length, plumule length and fresh weight of wheat seedlings under salinity stress. The authors attributed these effects to enhanced

water uptake and balanced nutrient absorption conferred by the extract.

Similarly, [28] demonstrated that a commercial seaweed extract improved germination percentage, seedling length and vigor in rice seeds exposed to drought stress. They proposed that the extract's growth-promoting hormones, antioxidants and osmolytes bolstered early seedling growth under water deficit conditions.

The beneficial effects of SPAe align with these studies and can be ascribed to its unique composition. *S. platensis* is rich in proteins, vitamins, minerals and bioactive pigments like C-phycoerythrin which exhibit antioxidant, anti-inflammatory and growth-stimulating properties [29]. These components likely attenuated cellular damage induced by drought and promoted optimal germination and early seedling establishment.

Interestingly, SPAe conferred positive effects even in the tolerant Sakha 95 cultivar, suggesting its potential to further enhance resilience across diverse genetic backgrounds. This is in agreement with findings by [30] where a brown algal extract improved germination in both drought-sensitive and tolerant wheat genotypes.

The study provides valuable insights into SPAe's efficacy as a biostimulant seed treatment for improving wheat germination and stand establishment under drought stress. Further research should explore optimal application rates and techniques. Elucidating SPAe's precise modes of action could uncover molecular markers for breeding more stress-resilient wheat varieties. Overall, algal biostimulants like SPAe show promise as sustainable tools to secure global wheat yields despite climate change-induced stresses.

The percent change in various germination parameters was calculated for each treatment relative to the control. For Shandawel 1, presoaking in SPAe (S1 + SPAe) reduced the drought-induced decrease in plumule length, radicle length, adventitious root number, and germination percentage compared to presoaking in distilled water (S1 + dist. H₂O). SPAe also decreased the drought-related losses in seedling fresh weight and water content. However, it increased seedling dry weight. In

Sakha 95, SPAE presoaking (S95 + SPAE) also mitigated the drought effects on plumule length, radicle length, adventitious roots, germination percentage, and seedling fresh weight and water content compared to distilled water (S95 + dist. H₂O). As in Shandawel 1, SPAE increased seedling dry weight under drought in S95.

Overall, SPAE exhibited alleviative effects on drought-induced reductions in early growth parameters, especially plumule and radicle growth and germination percentage. The extract also maintained fresh weight and water content but increased dry matter accumulation. The positive effects of SPAE align with recent studies on biostimulants for drought mitigation during germination. [31] reported that moringa leaf extract improved radicle and plumule growth, fresh weight, and germination percentage of wheat under osmotic stress. The authors highlighted the antioxidant compounds and osmoprotectants in the extract as key factors enhancing early vigor. Similarly, [32] found that brown algal extracts alleviated drought-induced reductions in plumule and radicle elongation in maize seedlings. They suggested the extracts promoted root growth, allowing better water uptake under limited water availability. The enhanced dry matter observed in our study parallels findings by [33], where biostimulants increased dry biomass in drought-stressed wheat and maize. They proposed that biostimulants enhance plant tolerance by modulating metabolic processes involved in growth and stress adaptation.

The present study thus corroborates emerging literature on the potential of biostimulants, including algal extracts like SPAE, to stimulate seedling establishment under drought. Further mechanistic studies should focus on deciphering the specific functional ingredients in SPAE that confer stress resilience during the critical germination phase in crops like wheat. Overall, integrating SPAE into cultivation strategies could provide sustainable solutions for securing optimal stand density and productivity in water-limited areas.

Conclusion

Based on the results, it was obvious that the grain presoaking in *Spirulina platensis* aqueous extract increase the drought tolerance in wheat

seedlings. The findings of this study contribute to the understanding of drought's severe constraints on agricultural production, particularly during the juvenile stage of plant growth. The results highlight the potential of *Spirulina platensis* aqueous extract as a novel means to mitigate the adverse effects of drought on wheat seedlings, emphasizing its practical applications in sustainable agriculture.

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