

# Role of nanosilicab to boost the activities of metabolites in *Triticum aestivum* facing drought stress

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## Research Article

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# Abstract

Drought stress is a threat to agriculture which is decreasing the yield of crops and creating a considerable loss. This research focused on the part played by silicon dioxide nanoparticles (SiO<sub>2</sub> NPs), biofertilizers, and nanosilicab on *Triticum aestivum* under control and drought stress. Nanosilicab enhanced the germination percentage, germination index, and germination vigor index by 23.07%, 14.49% and, 93.10% under control and 14.42%, 10.52%, and 46.15% under drought. In the pot experiment, the soil was treated with 150 mg/kg SiO<sub>2</sub> NPs, 1% biofertilizer and, 1% nanosilicab before sowing. Nanosilicab increased shoot length and root length by 9.39%, 10.76%, 22.41%, 18.76%, 30.58%, and, 21.56% under control and drought stress conditions. It also increased photosynthetic pigments, osmolytes content, relative water content, membrane stability index, phenol, and flavonoid content. The increase in antioxidant activity was significantly high by the application of nanosilicab i.e. the augmentation in catalase, peroxidase, and superoxide dismutase was 68.65%, 83.69% and, 85.99% respectively. It also increased the indole acetic acid and cytokinin to 22.28% and 14.79% in comparison to control. The improvement in hundred grain weight and grains per spike by the use of nanosilicab was 36.25%, 38.76%, 27.47%, and 22.59% as compared to control. The positive interaction of nanosilicab with the roots of plants in the rhizosphere improved the growth of plants significantly and a potential candidate for application on crops. The novelty of this study lies in the formulation of nanosilicab and its role in drought amelioration.

## Introduction

Drought stress is a global issue that damages the economy of different countries by imparting negative effects on crops. It was documented by Food and Agriculture Organization that in 2021 drought is a key driver causing food insecurity in the world (Fao, 2021). It's a natural hazard that reduces the chances of plant establishment. The first and foremost responses include the closure of stomata and reduced gaseous exchange. Drought stress enhances the number of reactive oxygen species (ROS) in cells a considerable increase was reported in the cellular compartments including chloroplast and mitochondria. When ROS is produced in low conc. they perform their role as signaling molecules to increase the flux of Ca<sup>2+</sup> and abscisic acid in the cells. While their high synthesis causes damaging effects on plants. They disrupt the structure of biomolecules like proteins, lipids, and DNA. They also decrease the membrane fluidity, enzyme activity, ions transport, inhibit protein synthesis and cause cell death (García-León et al., 2021; Sattar, Sher, et al., 2020). Drought stress delimits cell expansion, cell enlargement, cell division, differentiation and inhibits enzyme-catalyzed reactions. It also interrupts the process of photosynthesis which causes a decrease in biomass production and yield of crops. The decrease in the grain yield of *Triticum aestivum* by water deficiency was 51% whereas the total production was decreased by 20.6% (Dimkpa et al. 2020). The decrease in carboxylation, gaseous exchange, and rate of photosynthesis while the increase in lipid peroxidation, electrolyte leakage was also reported in six ecotypes of *T. aestivum* under water stress (Khalvandi et al. 2021). They also considerably decrease the growth of *Oryza sativa* (Ahmed et al. 2021a), *Medicago sativa* (Tyshchenko et al., 2020), *Beta vulgaris* (AlKahtani et al. 2021), *Nicotiana tabacum* (Begum et al. 2021), *Punica granatum* (Zahedi et al. 2021) and affect various other

crops. This situation reinforces the need to develop a suitable approach that decrease the damaging effects of water stress and improves the germination, establishment, and yield of crops.

The practice of nanoparticles application enhanced the resources using efficiency of plants and delimiting the chances of environmental pollution. The extensive and uncontrolled application of chemical fertilizer causes pronounced side effects on the human health and on ecosystem. Nanoparticles are more efficient due to their unique properties like high biosafety, bioactivity, and mobility. The application of silicon boost the growth of plants even in stress conditions hence silicon nanoparticles (SiO<sub>2</sub> NPs) should be explored for their potential in water stress (Ali et al. 2021; Khan et al. 2021).

Biofertilizer (BF) is another environment-friendly approach that was used in the agricultural sector for the improvement of crops. Application of BF increases the germination of *Zea mays* by 33.32% (Devi and Kumar 2020) and enhanced the production of bioactive compounds in *Coriandrum sativum* (Jiménez-Gómez et al. 2020). BF increases the stem diameter, number of leaves, and plant height in *Glycine max* (Miftakhurrohmat and Sutarman 2021). It acts as a soil cover and reduces the chances of nutrients and water loss. It decreases the cost of input, maintain soil health, and make them resilient against stressful conditions. It's a good alternative to chemical fertilizers and provides phosphorus, nitrogen, and growth hormones to plants (Shah et al. 2021).

The impact of SiO<sub>2</sub> NPs application on *T. aestivum* facing drought stress is still not investigated and there is a lack of knowledge about the appropriate concentration of SiO<sub>2</sub> NPs to be used on plants. There is a need to identify strains of plant growth-promoting rhizobacteria (PGPR) and to make efficient biofertilizers that may remarkably enhance the development of plants as compared to existing BF. There is also a research gap indicate that the role of nanosilicab should be evaluated. The experiments include formation of a fertilizer named as nanosilicab. The role of SiO<sub>2</sub> NPs, BF, and nanosilicab on *T. aestivum* was analyzed in drought stress and well-watered conditions.

## Materials And Methods

### Synthesis of Nanosilicab

Bacterial strains used in this experiment (include *Azospirillum brasilense*, *Bacillus* sp., and *Azospirillum lipoferum*) and SiO<sub>2</sub> NPs as reported in (Akhtar et al. 2021). The consortium of bacterial strains ( $1.13 \times 10^8$  CFU/mL) were prepared by mixing the equal volume of broth culture grown in incubator shaker at 28°C. The sample was centrifuged at 5000 rpm and cells were washed and suspended in phosphate buffer saline (OD 1 at 600 λ). A mixture was prepared by using 2% sodium alginate, starch (3 mg/mL), and SiO<sub>2</sub> NPs (150 mg/L). The consortium was added in the mixture at 2:1. The mixture was added drop wise in 2% calcium chloride solution by using sterile syringe. The beads of nanosilicab were separated by strainer and dried at 4°C. The viability of bacterial cells in the beads and soil were measured by plate count method as given by Panichikkal et al. (2021).

### Germination experiment

The effect of SiO<sub>2</sub> NPs, BF, and nanosilicab was analyzed on germination of *T. aestivum*. Seeds of *T. aestivum* (variety Pakistan-13) were sterilized by using 1% NaClO (Liu et al. 2021). Seeds were dipped in the freshly prepared BF, SiO<sub>2</sub> NPs solution (150 mg/L), and nanosilicab for 8 hrs, and control was dipped in distilled water (Kebrom et al. 2019). The seeds were placed on petriplates having a filter. Each treatment has 3 replicates and the experimental design was CRD (completely randomized design). Drought stress was applied on one set by using 10% PEG-6000 and the other set of petriplates were treated with distilled water. After 10 days of germination parameters including germination percentage, germination index, germination vigor index, seedling root length, seedling shoot length, seedling fresh and dry weight were measured. The completely randomized design was used and each treatment was replicated three times.

#### Experimental setup for the cultivation of plants

Healthy seeds of *T. aestivum* were selected for the pot experiment. Seeds collection and sterilization were as mentioned in the above section. The soil was taken from Arid Agriculture University Rawalpindi Pakistan and its analysis were carried out by using the protocol of Li et al. (2017) (Supplementary Table 1). The soil was treated with SiO<sub>2</sub> NPs (150 mg/kg), BF (70 mL/kg), and nanosilicab (3 beads/kg) and leave for 40 days. Plastic pots of medium size were filled with 5 kg soil and sowing was done in November. After 2 weeks of germination 4 plants were maintained in each pot by thinning. The design was completely randomized design each treatment has three replicates. When the plants were at the stem elongation stage drought was applied by maintaining 45% field capacity and after 20 days sampling was done.

#### Analysis of growth attributes

The length of plants (shoots and roots) was taken by using meter rod. The plants were uprooted and after removing dust their fresh weight was recorded immediately. The plants were placed in oven at 70°C to obtain dry weight (Danish et al. 2020).

#### Determination of chlorophyll and carotenoid content

Leaf samples were collected for measuring photosynthetic pigments. Leaves were homogenized in acetone (80%) and the absorbance of the filtrate was taken at 645, 663, and 470 nm by using spectrophotometer.

#### Determination of osmolytes content

To measure proline content leaf samples were blended with sulfosalicylic acid and the filtrate was collected. A mixture was prepared by adding equal volume of filtrate, ninhydrin reagent, and glacial acetic acid. The mixture was added in the test tube and after mixing with 4 mL toluene the upper layer was collected and its absorbance was taken at 520 nm. The standard curve was prepared by using proline (Bates et al. 1973). To measure sugar content leaf was homogenized with methanol (80%) and kept in

the water bath (70°C) for 30 min. Leaf extract (2 mL) was mixed with 4% phenol (2 mL) and sulphuric acid (2 mL) and absorbance was taken. The standard curve of glucose was prepared for calculation (Dubois et al. 1951).

#### Analysis of membrane stability and relative water content

The fresh leaves (FW) of leaves were taken immediately after sample collection. The leaf discs were dipped in distilled water for 24 hrs and their turgid weight was recorded (TW). The same samples were dried in oven at 70°C for 48 hrs and dry weight was measured. The following formula was used for the calculation

Relative water content (%) = [(Fresh weight – Dry weight)/Turgid weight] × 100 (Reza Morshedloo et al. 2017).

To measure membrane stability leaf (200 mg) was cut into pieces and placed in the test tube, distilled water was also added. They were kept in the water bath (40°C) for 30 min and the electrical conductivity (EC<sub>1</sub>) was recorded. They were again kept in the water bath (100°C) for 10 min and the electrical conductivity (EC<sub>2</sub>) was recorded. The following formula was used for calculation;

Membrane stability index (%) = [1 - (EC<sub>1</sub>/EC<sub>2</sub>)] × 100 (Rady et al. 2020).

#### Analysis of phenolic and flavonoids content

The sample was homogenized with 80% methanol and after centrifugation; the extract was treated with Folin-Ciocalteu reagent (0.5 mL) and 5% sodium carbonate (1 mL). The absorbance was taken (after 30 min) at 725 nm. Analysis of phenol content was done by using the calibration curve of Gallic acid. To measure flavonoids content leaf was homogenized with methanol. The extract (1 mL) was treated with 5% sodium nitrite (0.3 mL). The solution of aluminum chloride (0.3 mL of 3% solution) was also added after 5 min and after a further 5 min, 5 M sodium hydroxide (2 mL) was added. The final volume was adjusted to 10 mL by using distilled water. The absorbance was taken at 510 nm and calibration was done by using the standard curve of quercetin (Chavoushi et al. 2020).

#### Analysis of antioxidant production

Enzyme extract was prepared by grinding the leaves with 50 mM phosphate buffer in the presence of 1% polyvinylpyrrolidone and liquid nitrogen. The supernatant was used after centrifugation (15,000 g for 3 min) for the assay of antioxidant enzymes. To measure catalase activity enzyme extract (250 µL) was mixed with potassium phosphate buffer (50 mM), DH<sub>2</sub>O (450 µL), and hydrogen peroxide was used as substrate. The decrease in the absorbance was recorded for 3 min (Aebi 1984). Peroxide activity was determined by treating the reaction mixture (enzyme extract 10 µL, 20 µL of 100 mM guaiacol, 50 mM sodium acetate, 160 µL) with (10 µL) 100 mM H<sub>2</sub>O<sub>2</sub> and the increase in the absorbance was observed at 450 nm (Ullah et al., 2013). To measure superoxide dismutase activity reaction mixture was mixed with

riboflavin and kept under a fluorescent lamp (7 min). Blank was also prepared it contains the same solution but instead of enzyme extract extra buffer was mixed with the solutions and absorbance was taken at 450 nm (Giannopolitis and Ries 1977).

#### Determination of phytohormones

Analysis of Indole acetic acid, cytokinin, and abscisic was carried out in the leaves of *T. aestivum*. The sample was extracted by using methanol (80%) and incubated for 12 hrs at 4°C. It was centrifuged (15000 rpm) and after the collection of supernatant the process of extraction was repeated. Methanol was added to the supernatant and the content of hormones was measured by HPLC (Shimadzu CBM-20A Japan). Synthetic hormones (Sigma Chemical Co. USA) were used as standard (Lang et al. 2019).

#### Analysis of yield attributes

The spikes of wheat were collected after-ripening and the yield attributes including 100-grain weight and number of grains per spike were calculated (Shokat et al. 2020).

## Data analysis

Analysis of the data was performed by using Statistix 8.1 software. Analysis of variance was done and Tukey's test was applied to find the difference among mean values. The mean of treatments was significant. Standard deviation was also calculated and the data was presented in the form of tables and figures.

## Results

#### Evaluation of PGPR in nanosilicab

The viability of bacterial strains was tested after 20 days of nanosilicab formation. The growth of bacterial strains on agar plates was observed and they were identified by the analysis of their morphological characteristics. PGPR shows colony number in the range of 137–341 in different dilutions. It shows that bacterial viability was high in nanosilicab.

#### Germination attributes

Germination is the first stage of a plant's life cycle. In this experiment, the effect of SiO<sub>2</sub> NPs, BF, and nanosilicab was evaluated on germination attributes of *T. aestivum* (Table 1, 2). The use of SiO<sub>2</sub> NPs enhanced germination percentage, germination index and, germination vigor index to 16.78%, 8.33% and, 44.82% respectively. The increase in germination percentage, germination index and, germination vigor index by the application of BF was 21.43%, 10.50% and, 65.51% respectively in comparison to the untreated plants. The maximum values of these parameters were recorded in seeds treated with nanosilicab i.e. the improvement was 23.07% (germination percentage), 14.49% (germination index) and, 93.10% (germination vigor index).

Table 1

Influence of SiO<sub>2</sub> NPs, biofertilizer and, nanosilicab on germination attributes of *Triticum aestivum*.

Treatments	Germination percentage		Germination Index		Germination Vigor Index	
	non-stress	stress	non-stress	stress	non-stress	stress
Control	77.46 ± 0.29 <sup>c</sup>	41.10 ± 0.58 <sup>f</sup>	2.76 ± 0.00 <sup>d</sup>	1.33 ± 0.00 <sup>h</sup>	0.58 ± 0.00 <sup>d</sup>	0.13 ± 0.00 <sup>g</sup>
SiO <sub>2</sub> NPs	90.40 ± 0.20 <sup>b</sup>	43.66 ± 0.66 <sup>e</sup>	2.99 ± 0.00 <sup>c</sup>	1.38 ± 0.00 <sup>g</sup>	0.84 ± 0.00 <sup>c</sup>	0.15 ± 0.00 <sup>fg</sup>
Biofertilizer	94.06 ± 0.52 <sup>a</sup>	45.73 ± 0.26 <sup>de</sup>	3.05 ± 0.00 <sup>b</sup>	1.43 ± 0.00 <sup>f</sup>	0.96 ± 0.00 <sup>b</sup>	0.17 ± 0.00 <sup>ef</sup>
Nanosilicab	95.33 ± 0.33 <sup>a</sup>	47.03 ± 0.54 <sup>d</sup>	3.16 ± 0.00 <sup>a</sup>	1.47 ± 0.00 <sup>e</sup>	1.12 ± 0.00 <sup>a</sup>	0.19 ± 0.00 <sup>e</sup>

Table 2

Influence of SiO<sub>2</sub> NPs, biofertilizer and, nanosilicab on germination attributes of *Triticum aestivum*.

Treatments	Seedling shoot length (cm)		Seedling root length (cm)		Seedling fresh weight (g)		Seedling dry weight (g)	
	non-stress	stress	non-stress	stress	non-stress	stress	non-stress	stress
Control	6.66 ± 0.03 <sup>d</sup>	3.66 ± 0.03 <sup>h</sup>	6.36 ± 0.04 <sup>d</sup>	1.12 ± 0.00 <sup>g</sup>	0.21 ± 0.00 <sup>d</sup>	0.10 ± 0.00 <sup>f</sup>	0.07 ± 0.00 <sup>d</sup>	0.03 ± 0.00 <sup>fh</sup>
SiO <sub>2</sub> NPs	8.03 ± 0.01 <sup>c</sup>	4.26 ± 0.03 <sup>g</sup>	7.03 ± 0.01 <sup>c</sup>	1.37 ± 0.01 <sup>f</sup>	0.28 ± 0.00 <sup>c</sup>	0.11 ± 0.00 <sup>f</sup>	0.09 ± 0.00 <sup>c</sup>	0.04 ± 0.00 <sup>g</sup>
Biofertilizer	9.09 ± 0.07 <sup>b</sup>	4.50 ± 0.01 <sup>f</sup>	7.50 ± 0.02 <sup>b</sup>	1.46 ± 0.01 <sup>ef</sup>	0.31 ± 0.00 <sup>b</sup>	0.12 ± 0.00 <sup>e</sup>	0.11 ± 0.00 <sup>b</sup>	0.04 ± 0.00 <sup>f</sup>
Nanosilicab	9.85 ± 0.02 <sup>a</sup>	4.80 ± 0.02 <sup>e</sup>	8.43 ± 0.02 <sup>a</sup>	1.51 ± 0.00 <sup>e</sup>	0.35 ± 0.00 <sup>a</sup>	0.13 ± 0.00 <sup>e</sup>	0.12 ± 0.00 <sup>a</sup>	0.05 ± 0.00 <sup>e</sup>

Drought stress drastically affects the germination attributes of *T. aestivum*. The use of SiO<sub>2</sub> NPs improved the germination parameters but the use of BF was superior as compared to SiO<sub>2</sub> NPs i.e. the increase in germination percentage, germination index and, germination vigor index was 11.26%, 7.51% and, 30.76% respectively in comparison to untreated stress facing plants. The application of nanosilicab enhanced the germination percentage, germination index and, germination vigor index to 14.42%, 10.52% and, 46.15% respectively.

It was observed that SiO<sub>2</sub> NPs, BF, and nanosilicab enhanced the seedling length and biomass of *T. aestivum*. The exposure of seeds to SiO<sub>2</sub> NPs enhanced the shoot, root length, and fresh and dry weight of seedlings to 20.57%, 10.53%, 33.33% and, 37.32% respectively. The application of BF also imparts

good effects on seedlings while the use of nanosilicab gives the best results they increase shoot, root length, and fresh and dry weight of seedlings to 47.89%, 32.54%, 66.66%, 74.64%.

Drought stress causes a decline in the rate of seedling growth and biomass. The exogenous treatments of seeds with SiO<sub>2</sub> NPs improved the shoot length, root length, and fresh and dry weight of seedlings by 16.39%, 22.32%, 12.60% and, 17.14% respectively. The inoculation of BF enhanced these attributes significantly as compared to untreated ones under water stress. Nanosilicab works in collaboration with the plants metabolism and improved the shoot length, root length, and fresh and dry weight of seedlings to 31.14%, 34.82%, 31.60% and, 57.14% as compared untreated stress facing plants.

### Growth attributes

The use of SiO<sub>2</sub> NPs and BF shows positive effects on the development of *T. aestivum* (Fig. 1). They boost the growth of shoot length, and root length ad improved the fresh weight, and dry weight of plants. The increase in growth attributes was more prominent in plants receiving the application of nanosilicab as compared to other treatments. The improvement in shoot length, root length, fresh weight and, dry weight was 34.77%, 16.88%, 74.91% and, 45.83%.

Drought stress reduced the growth of *T. aestivum* significantly. The decrease in shoot length, fresh weight and, dry weight was 33.60%, 43.99% and, 40.62% respectively. The improvement in shoot length and root length was 9.39% and 10.76% by SiO<sub>2</sub> NPs and 22.41% and 18.76% by the inoculation of BF. The improvement in shoot length and root length by the use of nanosilicab was 30.58% and 21.56% respectively. SiO<sub>2</sub> NPs and BF improved the biomass of plants significantly ( $p < 0.05$ ). The augmentation in biomass was high (38.11% fresh weight and 26.01% dry weight) by BF as compared to SiO<sub>2</sub> NPs application. However the application of nanosilicab gives pronounced results they increase fresh weight and dry weight to 44.95% and 35.96% respectively as compared to stress facing (untreated) plants.

### Chlorophyll content and carotenoid content

Chlorophyll and carotenoids are photosynthetic pigments responsible for the growth of plants (Fig. 2). In this experiment, the plants grown in soil supplemented with SiO<sub>2</sub> NPs shows an increase of 19.01%, 13.20%, and 24.02% in chlorophyll a, chlorophyll b, and carotenoid content respectively. However, BF boosted the production of these pigments to 32.13% (chlorophyll a), 23.60% (chlorophyll b) and, 33.36% (carotenoid content). The application of nanosilicab imparts good effects on the physiology of plants. For instance, the increase in chlorophyll a (64.59%), chlorophyll b (48.80%) and, carotenoid content (34.23%) by the amendment of nanosilicab was high as compared to other treatments.

Exposure of *T. aestivum* to drought stress results in decreased amount of chlorophyll content and carotenoid content while the use of SiO<sub>2</sub> NPs and BF reduced the damaging effects of drought and revamp the physiology of plants. A marked increase was observed in chlorophyll a (18.93%), chlorophyll b (22.09%) and, carotenoid content (17.59%) when plants were cultivated in the soil inoculated with nanosilicab.

## Production of osmolytes

Production of osmolytes like proline and osmolytes were also recorded during this experiment (Table 3). It was observed that a high amount of proline and soluble sugar was synthesized in plants facing stress and SiO<sub>2</sub> NPs further increased the proline (16.48%) and soluble sugar content (20.30%) in *T. aestivum*. The plants were grown in the presence of BF when exposed to stress they increased the synthesis of proline and soluble sugar to 23.24% and 29.10% respectively. The use of nanosilicab boosts the synthesis and accumulation of proline and sugar to 29.45% and 35.47% respectively in comparison to untreated stress-facing plants.

Table 3  
Influence of SiO<sub>2</sub> NPs, biofertilizer and, nanosilicab on osmolyte content in *Triticum aestivum*.

Treatments	Proline content (µg/g FW)		Sugar content (mg/g FW)	
	non-stress	stress	non-stress	stress
Control	501.66 ± 1.20 <sup>h</sup>	740.33 ± 1.45 <sup>d</sup>	20.73 ± 0.37 <sup>e</sup>	32.30 ± 0.68 <sup>c</sup>
SiO <sub>2</sub> NPs	530.33 ± 2.02 <sup>g</sup>	862.88 ± 1.20 <sup>c</sup>	22.06 ± 0.83 <sup>de</sup>	38.86 ± 0.18 <sup>b</sup>
Biofertilizer	562.00 ± 1.73 <sup>f</sup>	912.66 ± 1.45 <sup>b</sup>	25.46 ± 0.49 <sup>d</sup>	41.70 ± 0.90 <sup>ab</sup>
Nanosilicab	607.00 ± 1.70 <sup>e</sup>	954.66 ± 2.40 <sup>a</sup>	26.10 ± 0.40 <sup>d</sup>	43.76 ± 1.80 <sup>a</sup>

## Relative water content (RWC) and Membrane stability index (MSI)

In control conditions membrane of tissues was stable and plants have high relative water content as well (Fig. 3). The application of SiO<sub>2</sub> NPs and BF further improved these attributes in *T. aestivum*. The alone treatment of SiO<sub>2</sub> NPs and BF improved relative water and membrane stability index by 24.76% (RWC), 11.11% (MSI), 27.16% (RWC), and 14.47% (MSI) respectively. While the use of nanosilicab also considerably enhanced the RWC (29.43%) and MSI (20.18%) in comparison to control.

Drought stress results in disruption of the cell membrane and lowers water content in plants. The improvement in RWC and MSI by the use of SiO<sub>2</sub> NPs was 15.83% and 6.63% respectively in comparison to untreated plants facing stress. The presence of BF improved these parameters to 20.95% and 15.89% respectively. The plants grown in the soil having nanosilicab shows an increase of 27.87% and 24.06% in RWC and MSI respectively.

## Phenols and flavonoids content

The phenol and flavonoids content was analyzed in this study and the data were presented in Table 4. It was noted that SiO<sub>2</sub> NPs enhanced the production of phenol and flavonoid to 22.17% and 14.56% respectively in well-watered plants. The use of BF further augmented the level of these constituents to

30.07% (phenol) and 20.84% (flavonoid) as compared to control. The phenol and flavonoid content in plants by the use of nanosilicab was 33.02% and 29.26% respectively.

Table 4  
Influence of SiO<sub>2</sub> NPs, biofertilizer and, nanosilicab on phenol and flavonoid content in *Triticum aestivum*.

Treatments	Phenol (mg/g FW)		Flavonoid (mg/g FW)	
	non-stress	stress	non-stress	stress
Control	31.69 ± 0.65 <sup>b</sup>	21.63 ± 0.53 <sup>d</sup>	0.48 ± 0.00 <sup>d</sup>	0.39 ± 0.00 <sup>h</sup>
SiO <sub>2</sub> NPs	38.71 ± 1.24 <sup>a</sup>	24.74 ± 0.57 <sup>cd</sup>	0.55 ± 0.00 <sup>c</sup>	0.41 ± 0.00 <sup>g</sup>
Biofertilizer	41.22 ± 0.72 <sup>a</sup>	26.83 ± 0.16 <sup>c</sup>	0.58 ± 0.00 <sup>b</sup>	0.43 ± 0.00 <sup>f</sup>
Nanosilicab	42.15 ± 0.84 <sup>a</sup>	27.82 ± 0.55 <sup>c</sup>	0.62 ± 0.00 <sup>a</sup>	0.45 ± 0.00 <sup>e</sup>

The data collected from plants facing drought stress indicate that SiO<sub>2</sub> NPs and BF enhanced the synthesis of phenols and flavonoids to some extent which played an important role in plants. The improvement in the content of phenol and flavonoid by the incubation of SiO<sub>2</sub> NPs and BF was 14.37% (phenol), 5.59% (flavonoid), 24.04% (phenol) and, 10.77% (flavonoid) in comparison to untreated one. The inoculation of nanosilicab gave best results as compared to the other treatments. The increase in phenol and flavonoid content was 28.64% and 16.28% respectively.

#### Antioxidant enzymes activity

Antioxidants perform scavenging mechanisms of reactive oxygen species and reduced their toxic effects on plants. The production of enzymatic antioxidants like catalase (CAT), peroxidase (POD) and, superoxide dismutase (SOD) were observed in this study and given in Table 5. The rate of antioxidants production was enhanced in plants facing drought stress. The increase in CAT, POD and, SOD in plants facing drought stress by SiO<sub>2</sub>NPs was 32.80%, 52.29% and, 57.53% respectively. The application of BF gives a better response as compared to SiO<sub>2</sub>NPs in plants. They enhanced the production of CAT, POD and, SOD to 47.15%, 64.46% and, 71.74% respectively in comparison to untreated stress exposed plants. The application of nanosilicab considerably improved the production of these antioxidant enzymes i.e. the increase was 68.65%, 83.69% and, 85.99% respectively.

Table 5

Influence of SiO<sub>2</sub> NPs, biofertilizer and, nanosilicab on antioxidant production in *Triticum aestivum*.

Treatments	CAT (unit/g FW)		POD (unit/g FW)		SOD (unit/g FW)	
	non-stress	stress	non-stress	stress	non-stress	stress
Control	51.16 ± 0.66 <sup>g</sup>	83.13 ± 0.56 <sup>d</sup>	65.26 ± 0.42 <sup>g</sup>	117.73 ± 0.64 <sup>d</sup>	102.86 ± 1.04 <sup>g</sup>	162.10 ± 1.09 <sup>d</sup>
SiO <sub>2</sub> NPs	56.60 ± 0.43 <sup>f</sup>	110.40 ± 1.21 <sup>c</sup>	81.90 ± 1.03 <sup>f</sup>	179.30 ± 0.47 <sup>c</sup>	126.83 ± 0.61 <sup>f</sup>	255.36 ± 1.25 <sup>c</sup>
Biofertilizer	64.23 ± 0.84 <sup>e</sup>	122.33 ± 0.88 <sup>b</sup>	93.80 ± 0.49 <sup>e</sup>	193.63 ± 0.4 <sup>b</sup>	134.83 ± 0.52 <sup>e</sup>	278.40 ± 1.78 <sup>b</sup>
Nanosilicab	66.43 ± 0.29 <sup>e</sup>	140.20 ± 1.11 <sup>a</sup>	96.23 ± 0.14 <sup>e</sup>	216.26 ± 0.63 <sup>a</sup>	141.26 ± 0.59 <sup>e</sup>	301.50 ± 2.51 <sup>a</sup>

### Phytohormones production

The rate of phytohormones production in *T. aestivum* was analyzed to evaluate the effect of SiO<sub>2</sub> NPs, BF, and nanosilicab application on indole acetic acid (IAA), cytokinin (CK), and abscisic acid (ABA) production in plants (Fig. 4). The increase in the synthesis of IAA and CK by the use of SiO<sub>2</sub> NPs and BF was 16.98%, 20.63%, 22.14%, and, 24.16%. While a decrease of 19.53% and 31.44% was observed in the level of ABA. The application of nanosilicab enhanced the IAA and CK levels to 24.93% and 28.10% and decrease ABA levels by 44.12% as compared to control.

The part played by SiO<sub>2</sub> NPs, BF, and nanosilicab in *T. aestivum* facing drought stress was significant at  $p < 0.05$ . They enhanced the level of phytohormones but the application of BF gave good results and the increase in IAA, CK, and decrease in the level of ABA was 17.96%, 11.99% and, 16.81%. The application of nanosilicab enhanced the synthesis of IAA and CK to 22.28% and 14.79% and also decreased the synthesis of ABA by 23.62% in comparison to untreated (stress facing) plants.

### Yield attributes

The effect of SiO<sub>2</sub> NPs and BF was also recorded on the yield attributes of *T. aestivum* (Table 6). The SiNPs application increased 100-grain weight and number of grains per spike to 14.80% and 23.86% respectively. The increment in the yield attributes by BF was 28.09% (100-grain weight) and 23.87% (grains per spike). The maximal values of yield were observed by the use of nanosilicab. The increase in 100-grain weight and grains per spike was 36.25% and 38.76% respectively.

Table 6  
Influence of SiO<sub>2</sub> NPs, biofertilizer and, nanosilicab on yield attributes of *Triticum aestivum*.

Treatments	100-grain weight (g)		Grains per spike	
	non-stress	stress	non-stress	stress
Control	3.31 ± 0.00 <sup>d</sup>	2.34 ± 0.00 <sup>h</sup>	34.50 ± 0.65 <sup>c</sup>	17.13 ± 0.59 <sup>e</sup>
SiO <sub>2</sub> NPs	3.80 ± 0.00 <sup>c</sup>	2.49 ± 0.00 <sup>g</sup>	37.16 ± 0.69 <sup>bc</sup>	19.40 ± 0.63 <sup>de</sup>
Biofertilizer	4.24 ± 0.00 <sup>b</sup>	2.63 ± 0.00 <sup>f</sup>	38.36 ± 0.32 <sup>b</sup>	20.53 ± 0.31 <sup>d</sup>
Nanosilicab	4.51 ± 0.00 <sup>a</sup>	2.83 ± 0.00 <sup>e</sup>	41.63 ± 0.60 <sup>a</sup>	21.00 ± 0.57 <sup>d</sup>

There was a decline in the yield of *T. aestivum* under drought stress. The use of SiO<sub>2</sub> NPs enhanced the yield attributes however the increase by the application of BF was significantly higher. The BF improved the 100-grain weight and grains per spike to 18.46% and 19.84%. While the increase in yield by the application of nanosilicab was 27.47% (100-grain weight) and 22.59% (grains per spike) respectively as compared to the untreated one.

## Discussion

The supplementation of SiO<sub>2</sub> NPs, BF, and nanosilicab delimits the negative effects of water stress and boost the germination and growth of *T. aestivum* in well-watered and stress conditions. They improved the germination of seeds their length and biomass as well. The improvement in germination may be linked with the lower ABA content by the use of these treatments as it creates seed dormancy under unfavorable conditions. These findings were similar as reported by Emamverdian et al., (2021) who reported that the application of SiO<sub>2</sub> NPs improved the germination percentage, germination rate, germination index and, vigor index of *Phyllostachys edulis* in stress conditions. Silicon NPs split the seed's dormancy and increased the germination from 81–88% in *T. aestivum* under stress conditions (Mushtaq et al., 2017). The application of BF increased the germination and seedling development in *Glycine max* and *Zea mays* (Kolhe and Barwant 2021). The BF may improve the physical and chemical properties of the medium which enhanced the germination of seeds. They increase the imbibition of water synthesize phytohormones and plant growth-promoting substances. The application of BF significantly increased the germination (86.11% ), seedling length, seedling girth, and biomass of *Embllica officinalis* (Veerappa Hongal et al. 2018). BF has a positive impact on seeds they improved the roots proliferation and chances of crop establishment in stress conditions (Dar et al. 2021). Nanosilicab have the dual properties and may enhance the germination of seeds by modulating hormonal changes and increasing the enzymatic activities involved in the metabolic processes during germination. He et al. (2017) study shows that encapsulated *Pseudomonas putida* enhanced the germination and biomass of *Oryza sativa* in saline conditions. The use of plant growth promoting encapsulated products was significant and they suppress the negative effects of environmental stresses.

In this study, the improvement in plant length and weight were may be due to the phytohormones production in plants including a high level of IAA and CK due to the interaction of nanosilicab in the plant's rhizosphere. These results were in line with Saberi-Rise & Moradi-Pour, (2020). They study the role of *Bacillus subtilis* coated with alginate and TiO<sub>2</sub> NPs on bean plants affected with *Rhizoctonia solani*. They documented that encapsulated *B. subtilis* inhibit the growth of *R. solani* and enhanced vegetative growth of plants significantly and it was due to the synthesis of metabolites like IAA. The application of NPs imparts stress resistance in plants and enhanced their rate of production. Silicon NPs regulates the metabolic activities in plants and improved the quality and quantity of crops (Abbasi Khalaki et al. 2021). Hussain et al., (2019) investigation show that the application of silicon NPs enhanced carotenoid content, chlorophyll b, chlorophyll a, stomatal conductance, rate of photosynthesis and, transpiration rate to 100%, 127%, 61%, 99%, 79% and, 84%. The treatment of Silicon NPs and BF improved the biomass of *Melissa officinalis* which was associated with the enhanced production of photosynthetic pigments, relative water content, and regulation of gaseous exchange attributes as well (Hatami et al. 2021). The inoculation of *Azospirillum lipoferum* and *A. brasilense* as a biofertilizer leads to sustainable crop production in agriculture. They can fix nitrogen, release osmolytes and phytohormones, enhanced nutrients uptake and detoxify the toxins in the rhizosphere of plants. They increase the yield of plants even under stress conditions by using their plant growth-promoting characteristics (Raffi and Charyulu 2021). These two bacterial strains were also used as BF in this study and gave better results. BF enhanced the nutrients uptake and translocation which results in high plant biomass production (Basu et al. 2021). The supplementation of BF increased the production of the fresh and dry weight of *Abelmoschus esculentus* by 50% in comparison with control (Bandopadhyay 2020).

It was documented that the supplementation of Si NPs and PGPR improved the physicochemical characteristics of soil and enzymatic activity which makes the soil more suitable for plant growth. They also increase the photosynthetic pigments, water content and, rate of photosynthesis which was the result of decreased oxidative stress. They also increased the antioxidant activities, proline content which reduces electrolyte leakage and ROS in the cells (Hafez et al. 2021). During this research, the use of SiO<sub>2</sub> NPs, BF, and nanosilicab improved the synthesis of photosynthetic pigments, proline, and sugar content in *T. aestivum* in stress and non-stress conditions. Silicon positively controls the production of ROS and reduces its damaging effects on the cells (Pereira et al. 2021). They maintain the water potential and osmotic potential in plants and conserve the water content in the cells which is required in various processes in the plant body (Sattar et al. 2020a). The soil and foliar use of Si NPs enhanced the chlorophyll, carbohydrates and, protein content in *Polianthes tuberosa* (Karimian et al. 2020). The combined incubation of SiO NPs and ZnO NPs boost the uptake of nutrients (P, K, and N), osmolyte production (sugar, proline), and antioxidant activities (POD, SOD and, CAT) in *Magnifera indica* in stress conditions. They also improved the quantity and quality of fruits and their nutritional value as well (Elsheery et al., 2020). The treatment of BF improved the development of plant including *Aloe vera* (Khajeeyan et al. 2019), *Coriandrum sativum* (Kadhim 2021), *Z. mays* (Abdel Latef et al. 2020), *Phaseolus vulgaris* (Chavoshi et al. 2018), and *T. aestivum* (Amna et al. 2019). The combined application of BF and arbuscular mycorrhizal fungi boost the growth of *Phoenix dactylifera* in a limited water supply.

They improved water potential, reduce electrolyte leakage, and maintains the photosynthetic apparatus which leads to improving the growth of plants. The elevated level of photosynthetic pigments (carotenoid and chlorophyll) and photosynthetic efficiency indicate the importance of these treatments on crops (Anli et al. 2020). The interaction of *Azospirillum*, *Azotobacter* and, mycorrhiza improved shoot and root dry weight, carotenoid content, proline production, potassium, and nitrogen uptake and oil content of *Valeriana officinalis* under drought stress (Ostadi et al. 2020). The collective use of salicylic acid and BF enhanced the photosynthetic pigments, osmolyte production, and activation of defense systems in plants facing water stress (Azmat et al. 2020). The combined use of BF and cycocel also creates positive responses in *T. aestivum* facing stress conditions. Hence the co-application of BF with beneficial substances is a better option to improve the growth of plants (Seyed Sharifi et al. 2017).

The stability of the cell membrane is one of the indicators that show the stability of plants under stress. In this study, the use of nanosilicab significantly increased membrane stability and relative water content in *T. aestivum*. The foliar treatment of SiO<sub>2</sub> NPs decreased electrolyte leakage to 4.93% in *Musa acuminata* in water deficit conditions. It indicates the decrease in membrane damage whose stability is important for the normal functioning of cells (Mahmoud et al., 2020). Si NPs also increased the water uptake and relative water content in *Rosa hybrida*. They retain the membrane integrity after harvesting by reducing lipid peroxidation and activation of antioxidant activities (El-Serafy 2019). The survey of the literature shows that the incubation of BF enhanced the growth of *Amaranthus tricolor* (Siswanti and Umah 2021), *Lectuca sativa* (Azarmi-Atajan and Sayyari-Zohan 2020), *Z. mays* (Gao et al. 2020), *Solanum lycopersicum* (Charles Oluwaseun et al., 2018), *Plantago ovata* and *Cassia alexandrina* (Singh et al. 2019). BF maintains the leaf chlorophyll content, relative water content, membrane stability index, and stomatal conductance in limited water availability (Mamnabi et al. 2020). The use of BF and FeO NPs decreased the ion leakage and maintain the permeability of cell membranes by improved scavenging activities of POD, CAT, polyphenol oxidase (PPO), and proline in *Z. mays* facing mild and severe water stress (Eliaspour et al. 2020). They also improved the physiological and biochemical traits of *Hordeum vulgare*. The increase in grain yield was also significant in stress and non-stress conditions (Dadashzadeh et al. 2018). The improvement in plant biomass and yield was also correlated with high water uptake and membrane stability of plants (Shiva et al. 2019).

Drought stress causes the high rate of free radicles production which disturbs the structure and function of cell membranes and organelles. Plants synthesize antioxidants to reduce the damage caused by these reactive oxygen species. They perform the scavenging activity of free radicles, neutralize ROS and regulate respiratory metabolism. They act as an electron donor and acceptor in the chloroplast and plasma membrane and cause resistance to oxidative stress. The level of detoxification of ROS is linked with the plant species, metabolic state, and the duration and intensity of drought stress. The antioxidant components are well distributed in the photosynthetic cells to protect their structure and function in stress conditions (Hasanuzzaman et al. 2018; Sharma et al. 2020). Several enzymes performed different functions in plants and the improvement in the activities of these enzymes was linked with the osmoprotectant and antioxidants production in plants during unfavorable conditions. Silicon performs a

defensive role in plants and helpful in limiting the negative effects of abiotic stress factors (Alzahrani et al. 2018). The analysis of data in this study shows that the inoculation of SiO<sub>2</sub> NPs and BF and nanosilicab enhanced the production of phenol, flavonoid, CAT, POD, and SOD in stress and un-stress conditions. Panichikkal et al. (2021) reported that nano-chitosan encapsulated *Bacillus licheniformis* decrease the stress symptoms in *Capsicum annuum* and improve growth of plants. Fatemi et al., (2021) reported that the use of Si NPs enhanced the level of flavonoid, vitamin C and, antioxidants in *Coriandrum sativum* in stress conditions. The reduction in hydrogen peroxide level and lipid peroxidation reaction was also associated with the application of Si NPs as they enhanced the formation of POD, SOD and, CAT in plants (Mukarram et al. 2021). The use of Si NPs regulates the ascorbate glutathione cycle in *Z. mays* and has ameliorative potential for plants in stress conditions (Tripathi et al. 2016). The combined use of Si NPs and Ti NPs increased the level of antioxidants as compared to their separate application in *Cuminum cyminum* in water stress (Salajegheh et al., 2020). The use of BF also gave positive results in this regard. For instance, the addition of BF and biochar increased the SOD and POD activity to 77.21%, 72.82%, 39.37%, 60.29% in leaves and roots of *Gossypium hirsutum* (Zhu et al. 2020). BF also increased the production of phenolic compounds in *Coriandrum sativum* (Jiménez-Gómez et al. 2020), catalase activity in *Capsicum annuum* (Gou et al. 2020), and glutathione activity in *Vicia dasycarpa* (Ahmadian et al. 2021).

Phytohormones are involved at different stages of plant development like apical dominance, cell division, cell elongation, tissue differentiation, flower development, fruit ripening, etc. During this study, the incubation of SiO<sub>2</sub> NPs, BF, and nanosilicab increased the production of indole acetic acid (IAA), cytokinin (CK) and, reduce the production of abscisic acid (ABA). These responses improved the growth and production of plants. ABA is a stress hormone it causes stomatal closure and slows down the metabolic processes in plants. Its low production is important to maintain the plant's life cycle (Ullah et al. 2018). The application of silicon regulate the level of protein and phytohormones expression in *Oryza sativa* (Jang et al. 2018). The combined application of Si, B, Zn and, zeolite nanoparticles increases the uptake of nutrients (N, Zn, P, B, K, and, Ca) and decreased the production of ABA in *Solanum tuberosum* under physiological drought (Mahmoud et al., 2019). PGPR has the potential to synthesize phytohormones and they also increased the synthesis of phytohormones in plants (Vishwakarma et al. 2018). Reported that PGPR has the potential to synthesize gibberellin and organic acids their inoculation improved the length and biomass of *Lectuca sativa* and *Brassica napus* (Kang et al. 2019). It was reported that auxin-producing PGPR enhanced the production of essential oil and the synthesis of secondary metabolites in aromatic and medicinal plants. They also improved the chemical composition of essential oils (Çakmakçı et al. 2020). The application of *Pseudomonas pseudoalcaligenes* enhanced the phytohormones, chlorophyll content antioxidant production and the activities of antioxidant enzymes in *Z. mays* facing drought stress (Yasmin et al. 2021).

The incubation of SiO<sub>2</sub> NPs and BF and nanosilicab in the soil increased the yield of *T. aestivum* in the current research. The use of nanosilicab gave more pronounced results. The review of literature shows that nanobased materials activate the stress related genes in plants to make them tolerant/resistant. The

application of nanomaterials suppress the expression of Cd transporter genes including OsLCT1, OsHMA3, and OsHNA2 in *Oryza sativa* facing Cd stress. They also enhanced the availability and assimilation of nutrients in plants which leads to better yield (Ahmed et al. 2021b). Ali et al., (2019) also documented that the use of Si NPs increases the yield of *T. aestivum* in stress conditions. The increase in biomass and grain yield of *Oryza sativa* by the application of Si and Se NPs was 50%, 38%, 27%, and 18% (Hussain et al., 2020). When plants receive Si supplements their cell membrane transporters which were responsible for the transport of Si become active and the influx of Si improves the chemical composition of plants. They regulate the structural characteristics of plants and enhanced the nutritional status of products (Asgari et al. 2018). The foliar spray of Si NPs improved ground cover, plant height, number of achenes/capitulum, and also the yield and yield component of *Carthamus tinctorius* (Janmohammadi et al., 2016). They also enhanced the carbon metabolism and improve the establishment of *Glycin max*. The increase in plant yield was 24.5% and 17.41% (Hussain et al., 2021). The application of BF also increases the seeds number and oil yield in *Brassica napus* besides improvement in morphological and physiological attributes in plants (Lally et al. 2017). The use of BF enhanced the synthesis of soluble sugar, proteins and, chlorophyll content by 2.04%, 5.93% and, 2.80% in *Brassica rapa* as compared to control (Ji et al. 2020). They also enhanced Ca, N, P, K, protein, and sugar content in plants (Raklami et al. 2019). The increase in fruit mass, the number of fruits per plant, and fruit yield was also augmented by the application of BF (Araújo et al. 2018).

## Conclusion And Recommendations

In the current study, the analysis of results revealed that the use of SiO<sub>2</sub> NPs, BF, and nanosilicab improved the characteristics of plants in stress and well-watered conditions. They enhanced germination, plant height, biomass, photosynthetic pigments, relative water content, and membrane stability. They also do the osmotic adjustment and enhanced the level of antioxidants to reduce oxidative damage. They maintain the level of phytohormones which plays a significant role in several mechanisms. The yield was also increased by the use of these treatments. Overall this study elucidates the positive response of *T. aestivum* towards the use of SiO<sub>2</sub> NPs, BF, and nanosilicab. The response of plants was better by the application of nanosilicab as compared to other treatments. It's a new product which suppresses the negative effects of drought stress. This novel approach could contribute in the advancement of agriculture sector.

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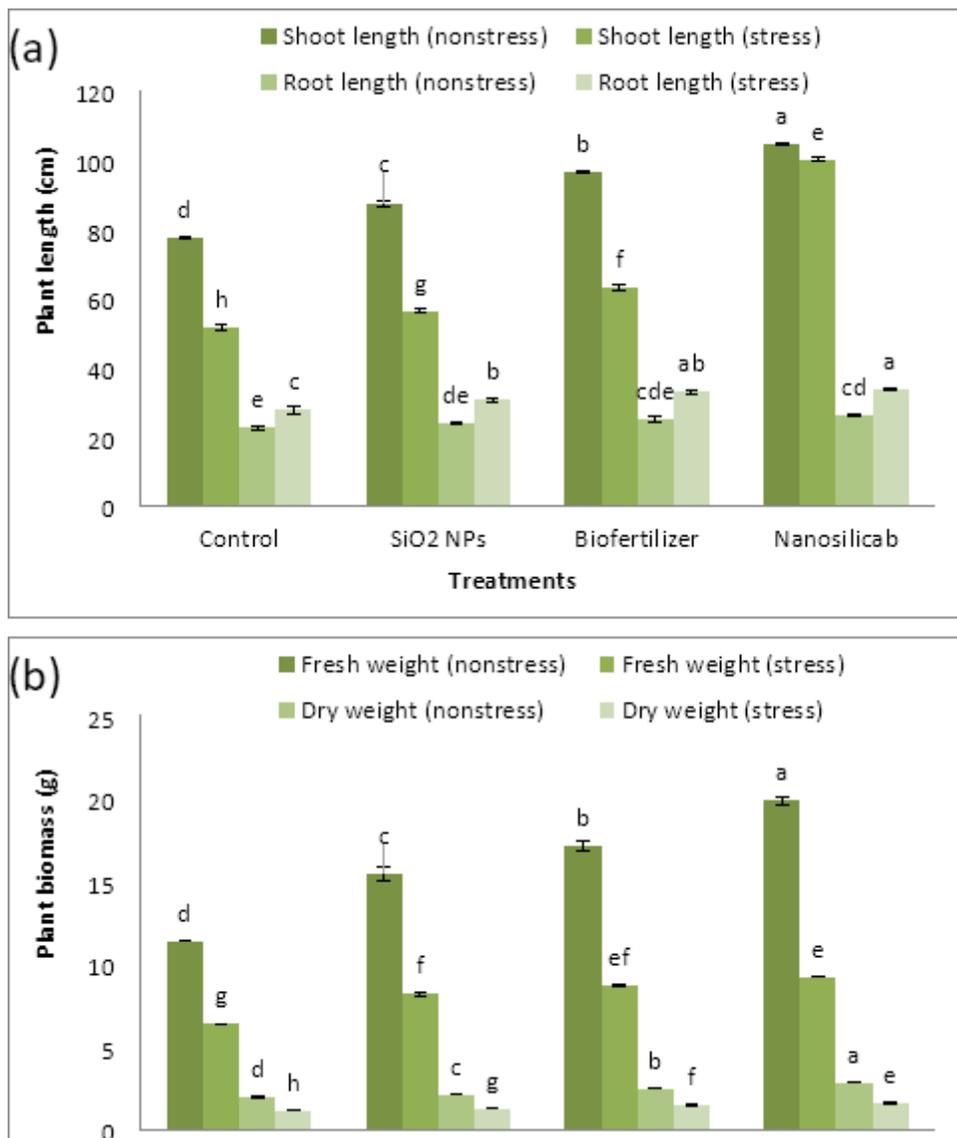
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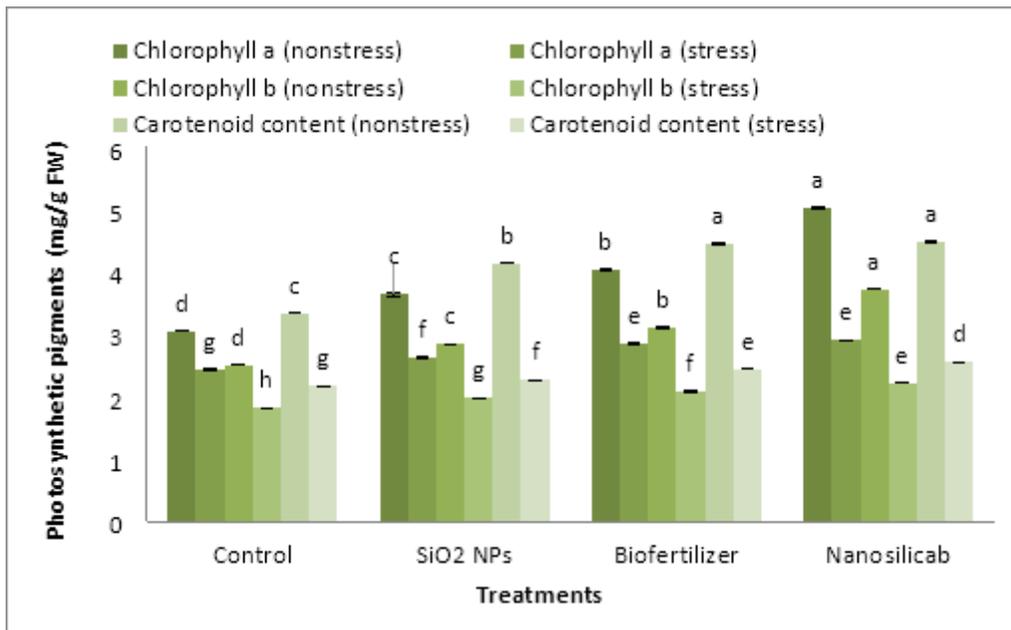
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## Figures



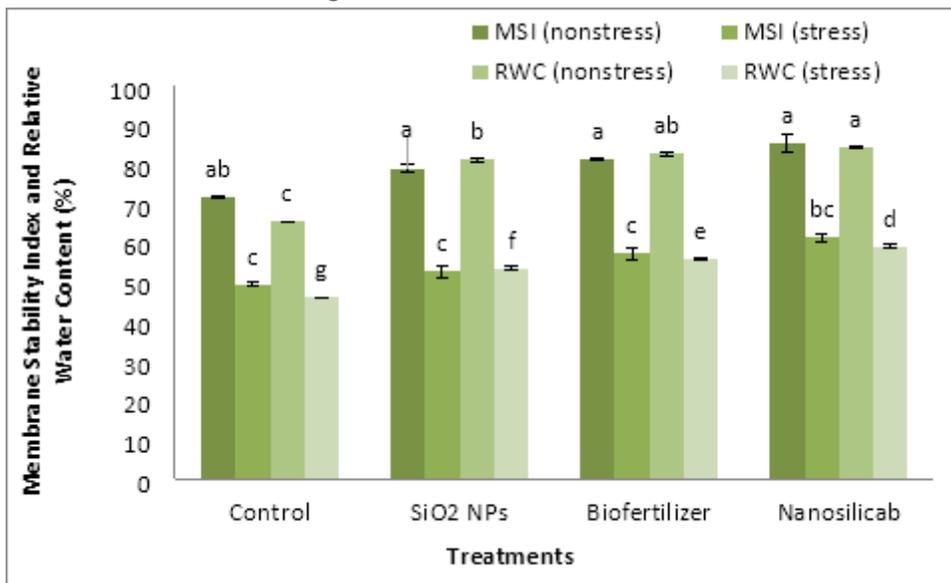
**Figure 1**

Influence of SiO<sub>2</sub> NPs, biofertilizer and, nanosilicab on length and biomass of *Triticum aestivum* in non-stress and stress conditions.



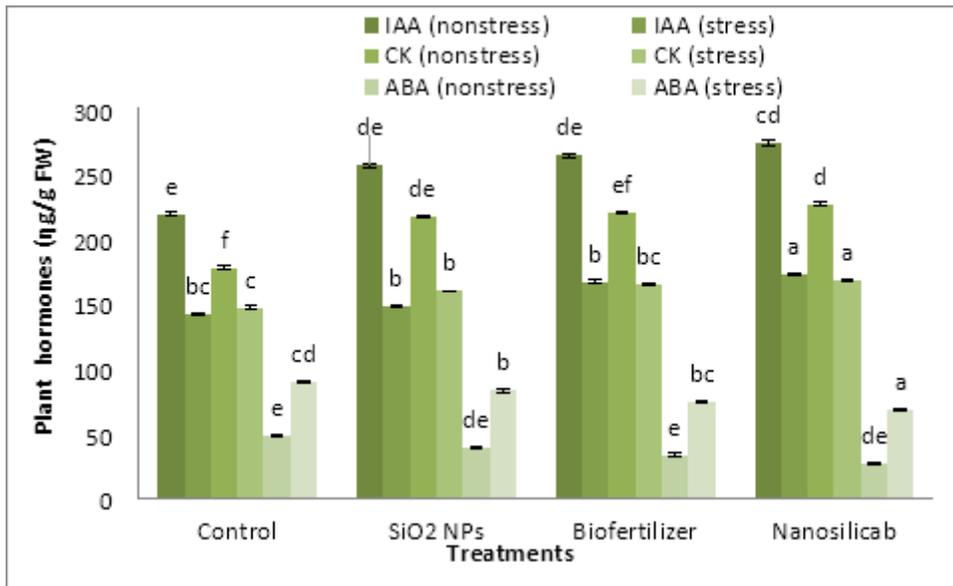
**Figure 2**

Influence of SiO<sub>2</sub> NPs, biofertilizer and, nanosilicab on photosynthetic pigments of *Triticum aestivum* under control and drought stressed conditions.



**Figure 3**

Influence of SiO<sub>2</sub> NPs, biofertilizer and, nanosilicab on relative water content and membrane stability index of *Triticum aestivum* under control and drought stressed conditions.



**Figure 4**

Influence of SiO<sub>2</sub> NPs, biofertilizer and, nanosilicab on hormonal content of *Triticum aestivum* in under control and drought stressed conditions.

## Supplementary Files

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