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

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**Abstract**

**Aims** This study was designed to assess the effect of nanosilicab fertilizer on *Triticum aestivum* under drought stress.

**Methods** The plants were grown in pots having the soil incubated with SiO$_2$ NPs, biofertilizer and nanosilicab. The experimental design was completely randomized design and drought stress was applied at stem elongation stage. Plants were maintained in the pots till the collection of yield.

**Results** 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 stress. In the pot experiment, the soil was treated with 150 mg/kg silicon dioxide nanoparticles (SiO$_2$), 1% biofertilizer and, 1% nanosilicab before sowing. Nanosilicab increased shoot length and root length by 34.77%, and 16.88% under control and 30.58%, and 21.56% under stress conditions, respectively. It also increased photosynthetic pigments, osmolytes content, relative water content, membrane stability index, phenol, and flavonoid content. The increase in antioxidant activity was significant 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 indole acetic acid and cytokinin by 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%, and 38.76% under control, and 27.47%, and 22.59% under stress conditions.

**Conclusion** The positive effect of nanosilicab on the roots of the plants improved the growth of plants significantly and this fertilizer showed potential for application on crops.

**Keywords** Silicon nanoparticles · Biofertilizer · Nanosilicab · Drought · *Triticum aestivum*

**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 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 early responses include the closure of stomata and reduced gaseous exchange. Drought stress enhances the number of reactive oxygen species (ROS) in cells and a considerable increase was reported in cellular compartments including the chloroplasts and mitochondria. When ROS is produced in low concentration, they perform their role as signaling molecules to increase the
flux of Ca\(^{2+}\) and abscisic acid in the cells. They disrupt the structure of biomolecules like proteins, lipids, and DNA. They also decrease membrane fluidity, enzyme activity, and ion transport, and inhibit protein synthesis, and cause cell death. (García-León and Standardi 2021; Sattar et al. 2020b). 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 *T. aestivum* by water deficiency was 51%, whereas the total production was decreased by 20.6% (Dimkpa et al. 2020). They decrease carboxylation, gaseous exchange, and rate of photosynthesis, while an increase in lipid peroxidation, electrolyte leakage was also reported in six ecotypes of *T. aestivum* under water stress (Khalvandi et al. 2021). They 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) and also affect various other crops. This situation reinforces the need to develop a suitable approach that decreases the damaging effects of water stress and improves the germination, establishment, and yield of crops.

The application of nanoparticles has been found to enhance the resource use efficiency of plants and decrease the chances of environmental pollution. The extensive and uncontrolled application of chemical fertilizer causes pronounced side effects on human health and on the ecosystem. Nanoparticles are more efficient due to their unique properties like high biosafety, bioactivity, and mobility. The application of silicon boosts the growth of plants even under stress conditions hence silicon dioxide nanoparticles (SiO\(_2\) NPs) should be explored for their potential in alleviating 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 increased 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 increased the stem diameter, number of leaves, and plant height of *Glycine max* (Miftakhurohmat and Sutarman 2021). It acts as a soil cover and reduces the chances of nutrients and water loss. It decreases the cost of input, maintains soil health, and makes plants 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).

Although the role of silicon dioxide nanoparticles (SiO\(_2\) NPs) and plant growth-promoting rhizobacteria (PGPR) is well established under drought, there is a need for testing their combined application, particularly in the form of a biofertilizer which can be easily applied. The experiments in this study include the formation of a fertilizer named nanosilicab and testing its efficacy for improving drought tolerance in wheat.

**Materials and methods**

**Synthesis of nanosilicab**

Bacterial strains (include *Azospirillum brasilense*, *Bacillus* sp., and *Azospirillum lipoferum*) and SiO\(_2\) NPs were used in this experiment, as previously reported in (Akhtar et al. 2021). The consortium of bacterial strains (1.13 \(10^{-8}\) CFU/mL) was prepared by mixing the equal volume of broth culture grown in an 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\(_2\) NPs (150 mg/L). The consortium was added to the mixture at 2:1. The mixture was added dropwise in 2% calcium chloride solution by using a 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 was measured by the plate count method as given by Panichkkal et al. (2021).

**Germination experiment**

The effect of SiO\(_2\) NPs, BF, and nanosilicab was analyzed on the 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\(_2\) NPs solution (150 mg/L), and nanosilicab for 8 h, and control was dipped in distilled water (Kebrom et al. 2019). The seeds were placed on Petri plates having filter paper. 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 Petri plates was 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 (Wang et al. 2020).

Experimental setup for the cultivation of plants

Healthy seeds of *T. aestivum* were selected for the pot experiment. Seeds collection and sterilization were done 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) (Table 1). The soil was treated with SiO$_2$ NPs (150 mg/kg), BF, and nanosilicab and was left for 40 days. Plastic pots of medium size (37 cm×28 cm) were filled with 5 kg soil and sowing was done in November. After two weeks of germination, four plants were maintained in each pot, by thinning. A completely randomized design (CRD) was used and each treatment had three replicates. When the plants were at the stem elongation stage (90 days old), 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 a meter rod. The plants were uprooted and after removing dust, their fresh weight was recorded immediately. The plants were placed in an oven at 70 °C to obtain dry weight (Danish et al. 2020).

**Table 1** Analysis of the soil sample used in the pot experiment

| Parameters          | Value                  |
|---------------------|------------------------|
| Electrical conductivity (ds/m) | 13.06 ± 0.23         |
| pH                  | 7.62 ± 0.07            |
| Moisture (%)        | 62.54 ± 2.75           |
| Organic matter (%)  | 0.36 ± 0.00            |
| N (mg/kg)           | 12.51 ± 0.01           |
| P (mg/kg)           | 10.31 ± 0.52           |
| K (mg/kg)           | 3.57 ± 0.63            |
| C (mg/kg)           | 2.68 ± 0.45            |

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 a spectrophotometer. The following equations were used to calculate chlorophyll and carotenoid content.

Chlorophyll a = 12.7 A663 - 2.7 A645
Chlorophyll b = 22.9 A645 - 4.7 A663 (Bruuinsma 1963).

Carotenoid = 1000 × A470 – 2.27 × chl. a – 81.4 × chl. b (Selvaraj 2018)

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 an equal volume of filtrate, ninhydrin reagent, and glacial acetic acid. The mixture was added to 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, the 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 were taken. The standard curve of glucose was prepared for calculation (Dubois et al. 1951).

Analysis of relative water content and membrane stability index

The fresh weight (FW) of leaves was taken immediately after sample collection. The leaf discs were dipped in distilled water for 24 h and their turgid weight was recorded (TW). The same samples were dried in an oven at 70 °C for 48 h and dry weight was measured. The following formula was used for the calculation (Reza Morshedloo et al. 2017):

\[ \text{Relative water content (%) = } \frac{\text{[Fresh weight–Dry weight]/Turgid weight]} \times 100. \]

To measure membrane stability, the leaf (200 mg) was cut into pieces and placed in the test tube, distilled water was also added. The whole setup was kept in the water bath (40 °C) for 30 min and the electrical conductivity (EC$_1$) was recorded. They were again kept in the water...
bath (100 °C) for 10 min and the electrical conductivity (EC2) was recorded. The following formula was used for calculation (Rady et al. 2020):

Membrane stability index (%) = \[1 - \left(\frac{EC_1}{EC_2}\right)\] × 100.

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, the 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 200 μL of potassium phosphate buffer (50 mM), DH2O (450 μL), and hydrogen peroxide (100 μL) 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 H2O2 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 and it contained the same solution but instead of enzyme extract, the 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 (IAA), cytokinin, and abscisic acid (ABA) was carried out in the leaves of *T. aestivum*. The sample was extracted by using methanol (80%) and incubated for 12 h at 4 °C. It was centrifuged (15,000 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.

Results

Evaluation of PGPR in nanosilicab

The growth of bacterial strains on agar plates was observed. Their colony number was in the range of 137–341, in different dilutions. It showed that bacterial viability was high in nanosilicab.

Germination experiment

In this experiment, the effect of SiO2 NPs, BF, and nanosilicab was evaluated on germination attributes of *T. aestivum* (Table 2). The use of SiO2 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 exposure of seeds to vum enhanced the seedling length and biomass of *Plant Soil* (2022) 477:99–115

| Treatments | Germination percentage (%) | Germination Index | Germination Vigor Index | Seedling shoot length (cm) | Seedling root length (cm) | Seedling fresh weight (g) | Seedling dry weight (g) |
|------------|---------------------------|------------------|------------------------|---------------------------|--------------------------|--------------------------|------------------------|
| T0         | 77.46±0.29<sup>c</sup>    | 2.76±0.00<sup>d</sup> | 0.58±0.00<sup>d</sup> | 6.66±0.03<sup>d</sup> | 6.36±0.04<sup>d</sup> | 0.21±0.00<sup>d</sup> | 0.07±0.00<sup>d</sup> |
| T1         | 90.40±0.20<sup>b</sup>    | 2.99±0.00<sup>c</sup> | 0.84±0.00<sup>c</sup> | 8.03±0.01<sup>c</sup> | 7.03±0.01<sup>c</sup> | 0.28±0.00<sup>c</sup> | 0.09±0.00<sup>c</sup> |
| T2         | 94.06±0.52<sup>a</sup>    | 3.05±0.00<sup>b</sup> | 0.96±0.00<sup>b</sup> | 9.09±0.07<sup>b</sup> | 7.50±0.02<sup>b</sup> | 0.31±0.00<sup>b</sup> | 0.11±0.00<sup>b</sup> |
| T3         | 95.33±0.33<sup>a</sup>    | 3.16±0.00<sup>a</sup> | 1.12±0.00<sup>a</sup> | 9.85±0.02<sup>a</sup> | 8.43±0.02<sup>a</sup> | 0.35±0.00<sup>a</sup> | 0.12±0.00<sup>a</sup> |
| T4         | 41.10±0.58<sup>f</sup>    | 1.33±0.00<sup>b</sup> | 0.13±0.00<sup>b</sup> | 3.66±0.03<sup>b</sup> | 1.12±0.00<sup>b</sup> | 0.10±0.00<sup>f</sup> | 0.03±0.00<sup>b</sup> |
| T5         | 43.66±0.66<sup>c</sup>    | 1.38±0.00<sup>f</sup> | 0.15±0.00<sup>f</sup> | 4.26±0.03<sup>f</sup> | 1.37±0.01<sup>f</sup> | 0.11±0.00<sup>f</sup> | 0.04±0.00<sup>f</sup> |
| T6         | 45.73±0.26<sup>abc</sup> | 1.43±0.00<sup>f</sup> | 0.17±0.00<sup>f</sup> | 4.50±0.01<sup>f</sup> | 1.46±0.01<sup>f</sup> | 0.12±0.00<sup>f</sup> | 0.04±0.00<sup>f</sup> |
| T7         | 47.03±0.54<sup>de</sup>   | 1.47±0.00<sup>e</sup> | 0.19±0.00<sup>e</sup> | 4.80±0.02<sup>e</sup> | 1.51±0.00<sup>e</sup> | 0.13±0.00<sup>f</sup> | 0.05±0.00<sup>e</sup> |

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).

Drought stress drastically affects the germination attributes of *Triticum aestivum*. The use of SiO<sub>2</sub> NPs improved the germination parameters but the use of BF performed better 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 length, root length, and fresh and dry weight of seedlings by 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 as they increased shoot, root length, and fresh and dry weight of seedlings to 47.89%, 32.54%, 66.66%, 74.64%.

Drought stress caused 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 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 to untreated stressed plants.

**Growth attributes**

The use of SiO<sub>2</sub> NPs and BF showed positive effects on the development of *T. aestivum* (Fig. 1). They boost the growth of shoot length, and root length and improve 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%, 16.88% 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. The increase in growth attributes was more prominent as compared to untreated stressed plants.

Chlorophyll content and carotenoid content

The effects of the various treatments on chlorophyll and carotenoids are shown in Fig. 2. In this...
In this experiment, the plants grown in soil supplemented with SiO$_2$ NPs showed 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 by 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 resulted in a decreased amount of chlorophyll content and carotenoid content while the use of SiO$_2$ NPs and BF reduced the damaging effects of drought. 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.

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**Fig. 1** Influence of SiO$_2$ NPs, biofertilizer and, nanosilicab on length and biomass of $Triticum$ $aestivum$ in non-stress and stress conditions. Treatments include, T0 = Control (well-watered), T1 = SiO$_2$ NPs (Well-watered), T2 = Biofertilizer (well-watered), T3 = Nanosilicab (well-watered), T4 = Control (drought), T5 = SiO$_2$ NPs (drought), T6 = Biofertilizer (drought), T7 = Nanosilicab (drought)

**Fig. 2** Influence of SiO$_2$ NPs, biofertilizer and, nanosilicab on photosynthetic pigments of $Triticum$ $aestivum$ under control and drought stressed conditions. Details of treatments as given in Fig. 1
Production of osmolytes

Production of osmolytes like proline was 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 SiO₂ NPs further increased the proline (16.48%) and soluble sugar content (20.30%) in **T. aestivum**. The plants grown in the presence of BF were 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.

Relative water content (RWC) and membrane stability index (MSI)

Under control conditions membrane of tissues was stable and plants have high relative water content as well (Fig. 3). The application of SiO₂ NPs and BF further improved these attributes in **T. aestivum**. The application of SiO₂ NPs improved relative water and membrane stability index by 24.76%, and 11.11% while BF improved these parameters by 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 resulted in disruption of the cell membrane and lowered water content in plants. The improvement in RWC and MSI by the use of SiO₂ NPs was 15.83% and 6.63% respectively in comparison to untreated plants facing stress. The presence of BF improved these parameters by 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 3. It was noted that SiO₂ 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.

The data collected from plants facing drought stress indicate that SiO₂ NPs and BF enhanced the synthesis of phenols and flavonoids which played an important role in plants. The improvement in the content of phenol and flavonoid by the incubation of SiO₂ NPs and BF was 14.37% (phenol), 5.59% (flavonoid), 24.04% (phenol) and, 10.77% (flavonoid) in comparison to the untreated one. The inoculation of nanosilicab gave the 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 reduce their toxic effects on plants. The production of enzymatic antioxidants like catalase (CAT), peroxidase (POD) and,

| Treatments | Proline content (μg/g FW) | Sugar content (mg/g FW) | Phenol (mg/g FW) | Flavonoid (mg/g FW) |
|------------|---------------------------|-------------------------|-----------------|---------------------|
| T0         | 501.66 ± 1.20h            | 20.73 ± 0.37e           | 31.69 ± 0.65b   | 0.48 ± 0.00d        |
| T1         | 530.33 ± 2.02g            | 22.06 ± 0.83de          | 38.71 ± 1.24a   | 0.55 ± 0.00c        |
| T2         | 562.00 ± 1.73f            | 25.46 ± 0.49d           | 41.22 ± 0.72a   | 0.58 ± 0.00b        |
| T3         | 607.00 ± 1.70e            | 26.10 ± 0.40d           | 42.15 ± 0.84e   | 0.62 ± 0.00a        |
| T4         | 740.33 ± 1.45d            | 32.30 ± 0.68h           | 21.63 ± 0.53d   | 0.39 ± 0.00n        |
| T5         | 862.88 ± 1.20c            | 38.86 ± 0.18b           | 24.74 ± 0.57d   | 0.41 ± 0.00g        |
| T6         | 912.66 ± 1.45b            | 41.70 ± 0.90ab          | 26.83 ± 0.16c   | 0.43 ± 0.00f        |
| T7         | 954.66 ± 2.40a            | 43.76 ± 1.80a           | 27.82 ± 0.55b   | 0.45 ± 0.00c        |
superoxide dismutase (SOD) was observed in this study and given in Table 4. 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 SiO2 NPs was 32.80%, 52.29% and, 57.53% respectively. The application of BF gave a better response as compared to SiO2 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.

Phytohormones production

The rate of phytohormones production in T. aestivum was analyzed to evaluate the effect of SiO2 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 SiO2 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 SiO2 NPs, BF, and nanosilicab in T. aestivum facing drought stress was significant at p < 0.05. They enhanced the level of phytohormones

| Treatments | CAT (unit/g FW) | POD (unit/g FW) | SOD (unit/g FW) | 100-grain weight (g) | Grains per spike |
|------------|----------------|----------------|----------------|----------------------|-----------------|
| T0         | 51.16 ± 0.66f  | 65.26 ± 0.42f  | 102.86 ± 1.04f | 3.31 ± 0.00d         | 34.50 ± 0.65c   |
| T1         | 56.60 ± 0.43f  | 81.90 ± 1.03f  | 126.83 ± 0.61f | 3.80 ± 0.00e         | 37.16 ± 0.69hc  |
| T2         | 64.23 ± 0.84e  | 93.80 ± 0.49g  | 134.83 ± 0.52e | 4.24 ± 0.00b         | 38.36 ± 0.32b   |
| T3         | 66.43 ± 0.29e  | 96.23 ± 0.14e  | 141.26 ± 0.59e | 4.51 ± 0.00a         | 41.63 ± 0.60a   |
| T4         | 83.13 ± 0.56d  | 117.73 ± 0.64d | 162.40 ± 1.04d | 2.34 ± 0.00b         | 17.13 ± 0.59c   |
| T5         | 110.40 ± 1.21c | 179.30 ± 0.47c | 255.36 ± 1.25c | 2.49 ± 0.00d         | 19.40 ± 0.63de  |
| T6         | 122.33 ± 0.88b | 193.63 ± 0.4b  | 278.40 ± 1.78b | 2.63 ± 0.00f         | 20.53 ± 0.31d   |
| T7         | 140.20 ± 1.11a | 216.26 ± 0.63a | 301.50 ± 2.51a | 2.83 ± 0.00c         | 21.00 ± 0.57d   |
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$_2$ NPs and BF was also recorded on the yield attributes of *T. aestivum* (Table 4). The SiO$_2$ NPs application increased 100-grain weight and the 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.

There was a decline in the yield of *T. aestivum* under drought stress. The use of SiO$_2$ 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$_2$ NPs, BF, and nanosilicab decreases the negative effects of water stress and boosts the germination and growth of *T. aestivum* in well-watered and stress conditions. They improved the germination of seeds, their length, and their biomass as well. Application of these treatments reduced the amount of ABA production which is one of the main causes of seed dormancy. These findings were similar as reported by Emamverdian et al. (2021) who reported that the application of SiO$_2$ NPs improved the germination percentage, germination rate, germination index and, vigor index of *Phyllostachys edulis* under stressed conditions. Silicon NPs break the seed’s dormancy and increase the germination from 81% to 88% in *T. aestivum* under stress conditions (Mushtaq et al. 2017). The application of BF increased the germination and seedling development in *G. max* and *Z. mays* (Kolhe and Barwant 2021). The BF may improve the physical and chemical properties of the soil which enhanced the germination of seeds. They increase the imbibition of water, synthesized 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 causes root proliferation and enhances the chances of crop establishment under stress conditions (Dar et al. 2021). Nanosilicab has 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 showed that encapsulated Pseudomonas putida enhanced the germination and biomass of O. 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 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 and Moradi-Pour (2020). They studied the role of Bacillus subtilis coated with alginate and TiO2 NPs on bean plants affected with Rhizoctonia solani. They documented that encapsulated B. subtilis inhibit the growth of Rhophitulus solani and enhance 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 the rate of production. Silicon NPs regulated the metabolic activities in plants and improved the quality and quantity of crops (Abbasi Khalaki et al. 2021). Hussain et al. (2019) investigation showed 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 A. lipoferum and A. brasilense as a biofertilizer leads to sustainable crop production in agriculture. They can fix nitrogen, release osmolytes and phytohormones, enhance nutrient 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 nutrient 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 SiO2 NPs, BF, and nanosilicab improved the synthesis of photosynthetic pigments, proline, and sugar content in T. aestivum in stress and control 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 boosts 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 plants including Aloe vera (Khajeeyan et al. 2019), C. 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 boosts the growth of Phoenix dactylifera in a limited water supply. They improved water potential, reduce electrolyte leakage, and maintain 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 SiO₂ NPs decreased electrolyte leakage to 4.93% in Musa acuminata in water deficit conditions. It indicated 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 retained 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 maintained 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 (Eliaispour et al. 2020). They also improved the physiological and biochemical traits of Hordeum vulgare. The increase in grain yield was also significant under stress and control 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 a high rate of free radicals 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 helps limit the negative effects of abiotic stress factors (Alzahrani et al. 2018). The analysis of data in this study shows that the inoculation of SiO₂ NPs and BF and nanosilicab enhanced the production of phenol, flavonoid, CAT, POD, and SOD in stress and unstress conditions. Panichikkal et al. (2021) reported that nano-chitosan encapsulated Bacillus licheniformis decrease the stress symptoms in Capsicum annum and improve the growth of plants. Fatemi et al. (2021) reported that the use of Si NPs enhanced the level of flavonoid, vitamin C and, antioxidants in C. 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 under 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 under 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 C. sativum (Jiménez-Gómez et al. 2020), catalase activity in C. annum (Gou et al. 2020), and glutathione activity in Vicia dasycarpa (Ahmadian et al. 2021). The use of BF regulate the expression of stress-responsive genes in plants which minimize the
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 SiO2 NPs, BF, and nanosilicab increased the production of indole acetic acid (IAA), cytokinin (CK) and, reduced 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 regulates the level of protein and phytohormones expression in O. sativa (Jang et al. 2018). The combined application of Si, B, Zn and, zeolite nanoparticles increased 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 increase the synthesis of phytohormones in plants (Vishwakarma et al. 2018). It has been reported that PGPR has the potential to synthesize gibberellin and organic acids and their inoculation improved the length and biomass of Lectuca sativa and Brassica napus (Kang et al. 2019). It was also 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. 2021b).

The incubation of SiO2 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 the literature shows that nano-based materials activate the stress-related genes in plants to make them tolerant/resistant. The application of nanomaterials suppressed the expression of Cd transporter genes including OsLCT1, OsHMA3, and OsHNA2 in O. 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 increased the yield of T. aestivum in stress conditions. The increase in biomass and grain yield of O. 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 enhance 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 (Jannomhammad 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 increased the seeds number and oil yield in B. 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 were also augmented by the application of BF (Araújo et al. 2018).

Conclusion and recommendations

The application of SiO2 NPs, BF, and nanosilicab showed positive effects on plant development and physiology. They enhanced germination, plant height, biomass, photosynthetic pigments, relative water content, and membrane stability. They also brought osmotic adjustment and enhanced the level of antioxidants to reduce oxidative damage. They maintained the level of phytohormones which played 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 SiO2 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 that suppresses the negative effects of...
drought stress. This novel approach may contribute to the advancement of the agriculture sector.

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