RESEARCH ARTICLE

Effect of silicon application with mycorrhizal inoculation on *Brassica juncea* cultivated under water stress

Ashutosh Srivastava[1], **Vijay Kumar Sharma**[2], **Prashant Kaushik**[3]*, **Mohamed A. El-Sheikh**[4], **Shaista Qadir**[5], **Sheikh Mansoor**[6]

1 Department of Botany, Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh,
2 Department Genetics and Plant Breeding, Banda University of Agriculture and Technology, Banda, Uttar Pradesh, India, **3** Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain, **4** Botany and Microbiology Department College of Science, King Saud University, Riyadh, Saudi Arabia, **5** Department of Botany, Womens College, Srinagar, Jammu and Kashmir, India, **6** Division of Biochemistry FBSc, SKUAST Jammu J&K, Jammu and Kashmir, India

* prashantkaushik104@gmail.com, prashantumri@gmail.com

Abstract

Brassica juncea L. is a significant member of the Brassicaceae family, also known as Indian mustard. Water is a limiting factor in the successful production of this crop. Here, we tested the effect of water shortage in *B. juncea* plants supplemented with or without the application of silicon and arbuscular mycorrhizal fungi in total 8 different treatments compared under open filed conditions using a randomised complete block design (RCBD). The treatments under control conditions were control (C, T1); C+Silicon (Si, T2); C+My (Mycorrhiza; T3); and C+Si+My (T4). In contrast, treatments under stress conditions were S (Stress; T5); S+Si (T6); S+My (T7) and S+Si+My (T8), respectively. In total, we evaluated 16 traits, including plant response to stress by evaluating peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) activity. The fresh weight (g) increased only 7.47 percent with mycorrhiza (C+My) and 22.39 percent with silicon (C+Si) but increased 291.08 percent with both mycorrhiza and silicon (C+Si+My). Using mycorrhiza (S+My) or silicon (S+Si) alone produced a significant increase of 53.16 percent and 55.84 percent in fresh weight, respectively, while using both mycorrhiza and silicon (S+Si+My) together produced a dramatic increase of 380.71 percent under stress conditions. Superoxidase dismutase concentration (Ug^{-1} FW) was found to be increased by 29.48 percent, 6.71 percent, and 22.63 percent after applying C+My, C+Si and C+Si+My, but treatment under stress revealed some contrasting trends, with an increase of 11.21 percent and 19.77 percent for S+My, S+Si+My, but a decrease of 13.15 percent for S+Si. Finally, in the presence of stress, carotenoid content (mg/g FW) increased by 58.06 percent, 54.83 percent, 183.87 percent with C+My, and 23.81 percent with S+My and S+Si+My, but decreased by 22.22 percent with S+Si. Silicon application proved to be more effective than AMF treatment with *Rhizophagus irregularis*, and the best results were obtained with the combination of Si and AMF. This work will help to suggest the measures to overcome the water stress in *B. juncea*.

Introduction

Brassica juncea (Czern) L. is an important member of the family *Brassicaceae*; it is also known as an 'Indian mustard (AABB, 2n = 36) [1, 2]. This is a common species of amphidiploids from a cross between *B. rapa* (AA, 2n = 20) and *B. nigra* (BB, 2n = 16) [3]. It is extensively cultivated in India, Canada, Australia, China and Russia [4, 5]. The economic importance attempts were made to raise their economic and agro-important characteristics such as oil quality, oil purity, seed scale, shattered pods and pathogen resistance. However, only a few papers covered the effects of water stress on important stages of *B. juncea* and how to eliminate it. However, *B. juncea's* water footprint is very small compared to most other Indian crops [6, 7]. Still, the emergence and sustainability of seedlings were severely hampered by severe water stress [8]. High temperatures and water tension during pod development are often considered to mitigate seed setting [9].

A major environmental factor limiting plant growth and crop quality is soil water availability [10, 11]. Water deficiency is due to surface water depletion (drought) or its absorption issue (physiological drought) [12]. Water is in the soil solution in this case. Still, plants cannot use it since there are certain physiological considerations such as elevated levels of salt (salinity), surplus water (floods) and low temperatures [13, 14]. Therefore, all of these factors influence water stress and changes in cell waters. Water potential decreases, and plant cell turgor decreases [15]. This interrupts most vital processes and reduces rates of production. Water deficit affects photosynthesis, absorption and transmission of essential nutrients and causes overproduction of ROS–species of reactive oxygen (O2-, 1O2, OH, H2O2) [16, 17]. These very reactive molecules cause extreme metabolic disorders and degradation of the membrane [18]. Silicon has been developed to reinforce cell walls and provide mechanical support to monocots and pteridophytes (under-understanding of dicots) by enhancing suberisation [19, 20].

Silicon (Si) application enhances the growth and increases plant tolerance to various abiotic stresses [19, 21, 22]. Soil content of Si can vary considerably between 1.0 and 45 percent dry weight [23]. While all plants have Si, Si levels differ substantially among species, ranging from 0.1 to 10% of the dry weight above ground [24, 25]. Monocots plants usually consume Si actively, whereas most dicots plants absorb Si passively [26, 27]. While Si is not an effective ingredient for higher plants, exogenous Si application has positively affected plant growth under abiotic stress [27, 28]. However, the advantages of Si are negligible or sometimes nonexistent except for stresses of some sort. Silicon treatment has been studied under water stress in some Si accumulators, like rice, maize, wheat and sorghum [29–31]. When Si is added, photosynthesis and associated carboxylase activities are increased under field drought conditions, as observed in wheat [32, 33]. In maize, Si addition can increase K and Ca levels, which indicates Si's essential role in plant mineral balance [34].

Glomus spp. spores such as *G. mosseae* and *G. intraradices* are the main root colonizers in saline soils [35, 36]. AM colonization enhances plant resistance to salinity, improves plant productivity, increases nutrient absorption [37], maintains ion equilibrium and facilitates water uptake [38]. Therefore, it would be important to study AM symbiosis's action in increasing the consumption of Si host plants and their cumulative position in salt tolerance. The hemicellulose of cell-walled connected with Si improved structural flexibility, evidently advantageous in water deficits [39, 40]. Furthermore, plant biological silicification, involving apoplastic polymerization of silicic acid, helps to form a silica barrier [41], which may reduce biotic and abiotic stress, prevent pathogenic contamination and penetration into plants potential toxicants such as aluminium (Al), manganese (Mn), cadmium (Cd), zinc (Zn) and sodium (Na) [42–44]. In root endodermis and exodermis, Silicon has helped in forming the Casparian band [20,

45]. Lignin and suberin based genes transcription were also activated [46]. These components can shape Na+ transport barriers in roots correlated with increased rice salt tolerance [47].

In this direction, Si's effects on transpiration vary with organisms and environmental factors. While Si inoculation increased transpiration in rice under both drought and salt-stressed conditions, it decreased the unstressed transpiration of rice [48]. The findings were similar in drought-stressed wheat and sorghum, while Si decreased transpiration in dry maize, and no effects were recorded on cucumber [20].

Such heterogeneity indicates divergent techniques among different plants, matching water absorption rates and water loss at the leaf stage. These methods and Si responses need further study and discovery. However, pathways that influence aquaporin's speech and function through the Si diet have yet to be resolved [49]. In addition to affecting hydraulic conductance and water transit by modulating aquaporin expression/activity, Si may impact water transport by modifying cell osmotic potential with enhanced osmolyte accumulation (e.g., proline, soluble sugars, inorganic ions, etc.) [50]. Therefore, the present study was carried out to study the role of Si application and mycorrhizal fungi for *B. juncea* plants cultivated with and without water stress.

Material and methods

Experimental layout

Field experimentation was carried out at Agriculture Research Farm, located at a latitude of 29˚95' North and longitude of 76˚82' from October to March of 2016–2017 at the temperature of 30±4˚C (day) and 20±5˚C (night). The plot was ploughed to make uniform topography using sandy-clayey loam soil employing a randomized complete block design with three replicates. Two irrigation regimes, including control (irrigation two times, one at 50% flowering stage and another one at 50% siliquae formation time), and total stress (no irrigation), were followed. B. *juncea* cultivar (RH-749) fertilized with recommended fertilizer dose of NPK for the treatment with silicon. Water stress was developed by withholding irrigation during the vital development stage (siliquae and flower initiation phases). The seeds were obtained from the oilseed section of CCS Hisar Agricultural University, Haryana. Physio-biochemical analysis of the experimental soil showed that it contained; sand 80.32%, silt 6.11%, clay 13.18%, organic matter 0.79%, total nitrogen (N) 110.15kg/ha, phosphorous (P) 7.59 kg/ha, K 439.61 kg/ha, and S 106.49 kg/ha. The pH of the soil was slightly basic at 7.9.

Silicon and arbuscular mycorrhizal fungi treatment

The salicylic acid solution was sprayed at a concentration of 150 ppm at the emergence, flowering, and siliqua stage of the *B. juncea* plants. Whereas *Rhizophagus irregularis* at a CFU count of 100 spores/g was procured from (M/S Shri Ram Solvent Extractions Pvt. Ltd., India). After mass multiplication, 100 g per plant is mixed with the field soil before transplanting.

There were eight treatments, i.e., 4 under regular irrigation and 4 under water stress. The treatments under control conditions were control (C, T1); C+Silicon (Si, T2); C+My (Mycorrhiza; T3); and C+Si+My (T4). Whereas treatments under stress conditions were, S (Stress; T5); S+Si (T6); S+My (T7) and S+Si+My (T8), respectively.

Plant characterization

The root to shoot length was measured on a meter scale, whereas whole plant height was estimated in cm at the blooming stage (day 63). The roots were then separated from the shoot, blotted, and subsequently weighed to record their fresh weight (FW) and then placed in an

oven at 80˚C overnight and weighed again to record the respective dry weight (DW). The leaf area was determined by utilizing a portable leaf area meter (Systronics 211, Ahmedabad, India), as per the manufacturer's instructions. Plants were harvested and taken for sampling from each plot. Leaves were removed from the stem, and fresh weight was noted (FW). Then they were oven-dried at 70˚C overnight for weighing dry weight (DW). This is done to evaluate Leaf Water Content using [(FW–DW)/FW] ×100.

The amount of chlorophyll present was determined by taking two wavelengths, i.e., 620 and 940 nm, with a CL-01 Chlorophyll Content Meter (Hansateh, Norfolk, UK). Whereas the method used to determine the membrane stability index (MSI) is defined elsewhere [19]. Using the sodium chloride reference standard, the osmotic potential was determined from the 3rd fully expanded leaf. Electrolyte leakage was measured by Dionisio-Sese and Tobita [51]. Furthermore, we evaluated the plant response to stress by evaluating peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) activity based on our previously defined protocol [52].

Data analysis

The data was collected from five plants that were comparable in appearance from each treatment, with the exception of the border plants. Each treatment's mean values were submitted to an analysis of variance (ANOVA) in order to identify differences among the treatments. Futher, the statistical significance of differences between treatment means was determined using Duncan's multiple range test (DMRT) for comparison of variance separated with (least significant difference) (LSD) as a post hoc test. All the statistical analysis were performed using JASP computer program (version 0.14.1.0). Whereas, the estimation of Pearson´s correlations coefficients was performed using the R platform (R Core Team 2015).

Results

Variation for characters

Variations in different traits of *Brassica juncea* were investigated after treatment with silicon and Mycorrhizal Inoculation under water stress and recorded in Table 1. The fresh weight was found to increase by only 7.47% and 22.39%, respectively, when treated with mycorrhiza (C +My) and silicon (C+Si) but increased drastically by 291.08% after treatment with both silicon and mycorrhiza (C+Si+My) (Table 1). The treatments were found to be more effective under conditions of stress which produced a significant increase of 53.16% and 55.84% in fresh

Table 1. Variation among the different treatments for morphological traits of *Brassica juncea* when treated with silicon and mycorrhizal inoculation of under water stress in the influence of silicon and mycorrhizal inoculation.

Treatments	Fresh weight (g)	Dry matter (g)	Root length (cm)	Leaf area (cm^2)	Plant height (cm)
C	22.33±1.53c	4.32±0.07d	16.67±0.65c	93.33±1.53cd	96.67±1.50c
C+My	24.00±1.00c	5,94±0.30c	19.30±1.00b	117.21±3.30b	104.67±1.48b
C+Si	27.33±0.85c	5.65±0.38c	17.17±0.96c	112.34±5.71b	105.67±1.71b
C+Si+My	87.33±4.51a	12.73±0.57a	21.71±1.02a	125.20±2.04a	112.03±1.95a
S	15.67±2.08d	2.96±0.08e	14.52±1.20d	71.33±0.58e	65.67±2.52f
S+My	24.00±2.00c	4.52±0.41d	16.67±1.35c	88.12±3.54d	73.67±2.52e
S+Si	24.42±1.06c	5.94±0.38c	19.32±1.45b	117.00±3.04b	104.67±1.76b
S+Si+My	75.33±5.77b	10.70±0.52b	18.67±0.65b	99.33±6.51c	89.67±1.83d

*Means within the groups are significantly different based on Duncan´s mean range test.

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weight when treated separately with mycorrhiza (S+My) and silicon (S+Si), respectively, and a dramatic increase of 380.71% when treated with both silicon and mycorrhiza (S+Si+My) (Table 1). Similarly, the dry matter content increased by 37.5%, 30.78%, and 194.67% with C+My, C+Si, and C+Si+My, respectively, and by 52.7%, 100.67%, and 261.48% with S+My, S+Si, and S+Si+My respectively (Table 1). Further, the root length exhibited a maximum increase of 30.23% with C+Si+My, followed by 15.77% with C+My, and 2.99% with C+Si. However, under conditions of stress, the treatment with silicon (S+Si) produced a maximum increase of 33.05% in the root length, followed by treatment with S+Si+My (28.58%) and S+My (14.8%) (Table 1).

In the case of leaf area, it was observed that silicon and mycorrhizal inoculation increased by 25.58%, 20.36%, and 34.14% with C+My, C+Si, and C+Si+My, respectively, while it recorded an increase of 23.54%, 64.02%, and 39.25% with S+My, S+Si, and S+Si+My respectively (Table 1). With regard to plant height, the increments recorded under normal conditions were 8.27%, 9.31%, and 15.89% with C+My, C+Si, and C+Si+My, respectively. In contrast, the corresponding values observed under water stress conditions were 12.18%, 59.38%, and 36.54% with S+My, S+Si, and S+Si+My, respectively (Table 1).

However, the percentage of relative water content did not change significantly; it decreased by 2.72% when treated with C+My but increased by 2.51% and 3.67% with C+Si and C+Si+My, respectively (Table 2). Further, it increased by 2.1%, 8.92%, and 8.43% after treatment with S+My, S+Si, and S+Si+My, respectively. Likewise, the percentage of membrane stability index increased nominally by 0.02%, 3.06%, and 6.27% under the influence of C+My, C+Si, and C+Si+My respectively, and by 2.6%, 10.37%, and 7.14% with S+My, S+Si, and S+Si+My respectively (Table 2). Although the electrolytic content increased by 1.51% after treatment with C+My, it decreased by 1.7% and 3.65% when treated with C+Si and C+Si+My, respectively (Table 2). Further, the maximum reduction in electrolytic content was observed with S+Si (13.45%), followed by S+Si+My (3.97%) and S+My (2.29%). In addition, the concentration of Superoxidase dismutase was found to be enhanced by 29.48%, 6.71%, and 22.63% after the application of C+My, C+Si, and C+Si+My, respectively, but treatment under stressed conditions revealed some contrasting trends, increase of 11.21% and 19.77% with S+My and S+Si+My respectively, and decrease of 13.15% with S+Si (Table 2).

Similarly, the concentration of peroxidase increased by 75.08%, 14.5%, and 30.39% with C+My, C+Si, and C+Si+My respectively, however, under stressed conditions, it increased by 28.93% and 39.56% with S+My and S+Si+My but decreased by 4.67% with S+Si (Table 2). Further, the ascorbate content, due to the introduction of silicon and mycorrhiza, increased by

Table 2. Variation among the different treatments for different stress tolerance indicator traits of *Brassica juncea* when treated with silicon along with mycorrhizal inoculation of under water stress in the influence of silicon and mycorrhizal inoculation.

Treatments	Relative water content (%)	Membrane stability index (%)	Electrolytic content (%)	Superoxidase dismutase (Ug^{-1} FW)	Peroxidase (Ug^{-1} FW)	Ascorbate (Ug^{-1} FW)	Catalase (Ug^{-1} FW)
C	81.38±0.15b	66.89±1.28c	24.58±1.06e	14.89±2.27e	9.31±5.80d	5.14±2.13c	3.88±1.66e
C+My	78.66±1.50c	66.91±1.20c	26.09±0.57d	19.28±3.07d	16.30±3.60bc	9.43±1.70bc	7.08±1.63cd
C+Si	83.89±1.27a	69.95±0.77b	22.88±0.65f	15.89±2.64e	10.66±5.86d	5.96±1.57c	4.57±1.30de
C+Si+My	85.05±1.34a	73.16±1.04a	20.93±0.75g	18.26±3.43d	12.14±3.91cd	7.27±2.31c	5.66±1.65de
S	69.74±0.46e	56.54±1.76f	39.54±0.81a	22.2±4.04c	17.11±5.84b	9.83±1.22bc	9.15±1.13bc
S+My	71.84±0.52d	59.14±1.50e	37.25±0.87b	24.69±3.51b	22.06±3.28a	14.33±4.32ab	11.86±0.70b
S+Si	78.66±1.54c	66.91±1.21c	26.09±0.57d	19.28±3.07d	16.31±3.60bc	9.43±1.75bc	7.08±1.72cd
S+Si+My	78.17±0.48c	63.68±1.45d	35.57±0.70c	26.59±2.91a	23.88±3.16a	15.83±5.02a	15.35±2.18a

*Means within the groups are significantly different based on Duncan´s mean range test.

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Table 3. Variation among the different treatments for leaf pigments of *Brassica juncea* when treated with silicon along with mycorrhizal inoculation of under water stress in the influence of silicon and mycorrhizal inoculation.

Treatments	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoids (mg/g FW)
C	2.91±0.14b	1.03±0.09a	3.93±0.23b	0.31±0.08e
C+My	2.67±0.06c	0.88±0.02b	3.55±0.07c	0.49±0.02de
C+Si	3.02±0.20ab	1.05±0.10a	4.08±0.30ab	0.48±0.03de
C+Si+My	3.17±0.15a	1.11±0.13a	4.28±0.30a	0.88±0.02b
S	2.08±0.18e	0.65±0.10c	2.73±0.06e	0.63±0.05cd
S+My	2.29±0.03d	0.79±0.03b	3.08±0.07d	0.78±0.03bc
S+Si	2.67±0.06c	0.88±0.02b	3.55±0.06c	0.49±0.12de
S+Si+My	2.38±0.07d	0.80±0.03b	3.18±0.12c	1.56±0.04a

*Means within the groups are significantly different based on Duncan´s mean range test.

83.46%, 15.95%, and 41.44% with C+My, C+Si, and C+Si+My respectively, while it increased by 45.77% with S+My and 61.03% with S+Si+My, but decreased nominally by 4.07% with S+Si (Table 2). The catalase content recorded increments of 82.47%, 17.78%, and 45.87% when treated with C+My, C+Si, and C+Si+My respectively, however, when observed under water stress, it exhibited an increase of 29.61% after treatment with S+My and 67.76% with S+My +Si, but a decrease of 22.62% with S+Si (Table 2).

Finally, the total chlorophyll content (chlorophyll a and chlorophyll b) produced no remarkable variations. However, the carotenoid content indicated a considerable increase when introduced with both silicon and mycorrhiza (Table 3). The total chlorophyll content was reduced by 9.67% when treated with C+My, while enhanced by 3.81% and 8.90% with C +Si and C+Si+My respectively, however, when investigated under stress, it was found to increase by 12.82%, 30.03%, and 16.48% with S+My, S+Si, and S+Si+My respectively (Table 3). The carotenoid content increased by 58.06%, 54.83%, and 183.87% with C+My, C +Si, and C+Si+My respectively, while under conditions of stress, it increased by 23.81%, and 147.62% with S+My, and S+Si+My respectively, but decreased by 22.22% with S+Si (Table 3).

Correlations

There were 136 correlations in total, but only 77 were found to be significant. Out of these significant correlations, 11 correlations were absolute (>0.9) (Fig 1). Dry matter was positively correlated with fresh weight (0.977), root length (0.792), leaf area (0.62), plant height (0.569), relative water content (0.682), membrane stability index (0.69), catalase concentration (0.49), chlorophyll a (0.513), chlorophyll b (0.469), total chlorophyll content (0.501), carotenoid content (0.832) (Fig 1). Further, the root length showed positive correlation with fresh weight (0.682), leaf area (0.877), plant height (0.8), relative water content (0.684), membrane stability index (0.841), superoxidase dismutase (0.453), chlorophyll a (0.655), chlorophyll b (0.577), total chlorophyll content (0.632), carotenoid (0.473).

The plant height was found to be positively correlated with relative water content (0.844), membrane stability index (0.931), chlorophyll a (0.759), chlorophyll b (0.625), and total chlorophyll content (0.721) (Fig 1). Further, osmotic potential exhibited a significant correlation with different traits, although in negative direction, dry matter (-0.452), root length (-0.707), leaf area (-0.864), plant height (-0.945), relative water content (-0.843), membrane stability index (-0.933), chlorophyll a (-0.883), chlorophyll b (-0.777), and total chlorophyll content (-0.856), except for electrolytic content (0.981) where it showed positive correlation (Fig 1). Besides, the concentration of superoxidase dismutase marked direct correlation with fresh
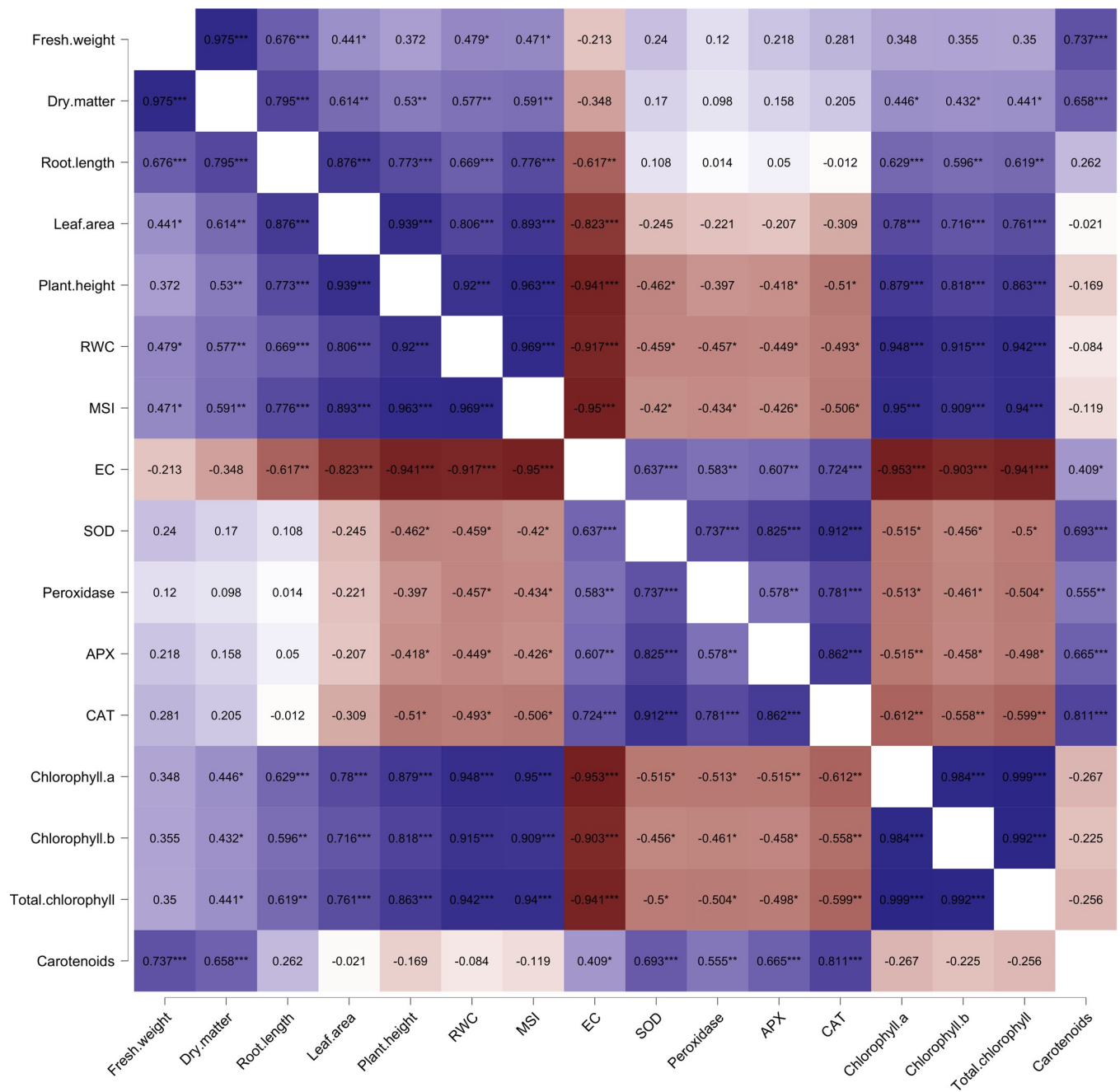
Fig 1. Correlations among the sixteen traits studied for *B. juncea* plants cultivated under water stress in the influence of silicon and mycorrhizal inoculation.

weight (0.418), peroxidase (0.56), ascorbate (0.697), catalase (0.836), and carotenoid (0.599) (Fig 1).

However, the peroxidase concentration showed a significant correlation only with catalase (0.619), in addition to superoxidase dismutase. In case of ascorbate concentration, it was observed that there were considerable positive correlations with catalase (0.752) and carotenoid (0.557). At the same time, catalase concentration was also correlated with fresh weight (0.519) and carotenoid content (0.796), besides other traits mentioned above (Fig 1). Finally,

the fresh weight, in addition to the traits discussed above, marked correlations with total chlorophyll content (0.44) and carotenoid content (0.877) also, both in a positive direction (Fig 1).

Discussion

In arbuscular mycorrhizal fungi colonized plants, AMF symbiosis increased biomass and other morphological traits of *B. juncea* [53]. Arbuscular mycorrhizal fungi contributed to the overall better performance of the plants. The biomass production and pigment concentrations decreased under the stress conditions. Silicon and arbuscular mycorrhizal fungi treatment have elevated the symptoms of water stress and improved the quality of leaf water and photosynthesis, which have led to increased biomass growth. Silicon and arbuscular mycorrhizal fungi have been applied to increase the net photosynthesis rate by increasing stomach behavior. This growth can be attributed to better net CO2 assimilation and appropriate distribution of photosynthates that can stimulate root production in such circumstances [54, 55]. In the *B. juncea* plants, the combination of organic osmolytes, which contribute to osmotic gradients in the atmosphere, has been observed as a typical response to water stress.

However, osmo-adjustment did not mediate AMF and Si's alleviatory effect and organic osmolyte levels decreased in +AMF and +Si plants [56, 57]. These results show that the Si-mediated increase in leaf water use was not due to a rise in water-stressed strawberry factories' osmotic motive power. There was an increase in leaf's relative water content (RWC) by increased water absorption capacity, which, in essence, prevented stomach closure and retained an excellent photosynthetic ability to support growth and the supply of dry matter [58, 59]. Rising volumes of antioxidant enzymes are usually expected to mitigate the stress. Arbuscular mycorrhizal fungi and silicon's stress reduction solution may also be less expensive to increase water absorption than osmo-modification strategies. This discovery compares with our previous observation on tobacco plants that has revealed a Si-medium shift in plant water status by adding organic osmolytes, including soluble sugars, free amino acids and proline [60, 61].

Comparing arbuscular mycorrhizal fungi and silicon treatments to strawberry leaves revealed a specific strategy for changing the roots' arbuscular mycorrhizal fungi and silicon water economies [62]. In tomatoes, the root osmotic potential was not altered in Si-treated plants [63]. In cucumbers, the role of osmotic motive force in Si-mediated water uptake was genotype-dependent [64]. These results collectively suggested optimizing water quality and capacity for osmotic stress-dependent Si-treated plants based on plant species, organisms and genotypes.

In plant-mycorrhizal interactions, nutrients are retained, and plant development is enhanced. Arbuscular mycorrhizal fungi hyphal networks and glomalin secretion help soil absorb water and nutrients [65–67]. Moreover, arbuscular mycorrhizal fungi can develop drought-adaptive strategies utilizing radical extra-hyphae and affect plant processes such as photosynthesis, root conductivity and root architecture [68, 69]. Arbuscular mycorrhizal fungi -mediated response is a multi-faceted process of drought-responsive gene expression and activation [70]. These metabolic compounds reduce the osmotic ability and hence leaf water capacity in plants subjected to drought. Arbuscular mycorrhizal fungi plants overcome oxidative stress triggered by a deficiency of water by promoting antioxidant compounds to scavenge ROS and facilitate enzyme antioxidant activities [71, 72]. Arbuscular mycorrhizal fungi root colonization enhances root formation, hydraulic properties and root design, resulting in a highly efficient root system for water nutrients absorption [73]. In a previous report, *G. mosseae* and *G. deserticola* demonstrated improved infectivity when AMF spores were treated by

storages in various soil water capacities [74, 75], implying that their consistency in root colonization could have good impacts.

Arbuscular mycorrhizal fungi has shown correct water intakes and improves plant nutrition by hyphal elongation in a drought stress analysis. Besides, enhanced water status will contribute to increased root and hydraulic conductivity. Arbuscular mycorrhizal fungi perseverance to regenerate a linked network after facing a water deficit stress, particularly anastomosis to the disrupted mycelium [76, 77].

Silicon, notably water deficits, has shown positive results on agriculture to mitigate plant-inherent abiotic tensions. This is because of its polymerization in the cell walls of roots, stems and leaves after absorption and accumulation, which creates a double layer of silica [78, 79]. Thus, an enzyme similar to plant protection mechanisms improves the cell walls' strength and steepness, decreases perspiration, and increases peroxidase activity. Under field conditions, Si facilitated greater dehydration tolerance and turgor loss by preserving the water content in cells and increasing photosynthesis production [80, 81]. The mechanism of abiotic stress tolerance in Si influenced several researchers investigated plants. Silicon's usage increases plant resistance to water deficits. Spatial benefits to dry plants may be attributed, in part, to its positive impact on the water state and photosynthesis of plants. It has been verified that Si also affects inorganic phosphorus leaf concentration [33, 82]. Proline is an amino acid that avoids the drought and tension in plants due to salinity [83], retains osmotic changes [84], metabolises antioxidants [85], modulates reactive oxygen species [86], and preserves the cell membrane's stability [87]. Mauad, Crusciol [88], Raza, Haider [89], however, finding that a proline concentration is increased in wheat leaves, triggering symptoms alongside the plant and not as a source of resistance to water stress incorporating silicon decreases proline accumulation. A lower degree of chlorophyll or unchanged water stress for other animals was recorded depending on the water deficit and severity.

Similarly, the reduction in proline concentrations induced by AMF suggests that AMF colonization mitigated water stress. The impaired production of amino acid proteins was detected under leaf water stress and measured by a concomitant accumulation of free AA with a reduced protein concentration. The protein storage allows the plant to sustain the water level of leaves [90], mitigate the harmful effects of active and reactive oxygen species in severe and long-term drought [91], and preserve leaves' water status. In the leaves and roots, water stress releases antioxidant enzymes. However, this activation was insufficient to protect plants against ROS, which is well manifested in increasing concentrations of MDA in water-stressed. The application of AMF and Si to the plants has also increased the antioxidant defense enzyme's operation (particularly of SOD) [92, 93]. In stressed plants, however, there is no direct biochemical connection between Si and antioxidant capacity. Biochemical improvement of antioxidant resistance pathways was considered a desirable physical result of cell membrane Si-deposition [32, 94]. Some researchers contend that the implication of Si in plant metabolism is caused by Si-induced improvements in antioxidant enzyme activity and amounts of non-enzymatic antioxidant substances in plants subjected to abiotic stress [42, 95].

Conclusions

Brassica juncea L., often known as Indian mustard, is a well-known member of the Brassicaceae family of plants. The availability of water is a significant constraint on the cultivation of this crop. We examined the effects of water shortage on *B. juncea* plants with and without silicon and arbuscular mycorrhizal fungus in 8 different treatments in an open field environment (RCBD). The plant reaction to stress was measured using peroxidase, superoxide dismutase, and catalase activities. The best outcomes were obtained from combining Si and AMF under

normal as well as under stress conditions. With this information a new water saving technology for *B. juncea* may be developed.

Supporting information

S1 File.
(XLSX)

Acknowledgments

Author Contributions

Conceptualization: Ashutosh Srivastava, Prashant Kaushik.

Data curation: Ashutosh Srivastava.

Formal analysis: Vijay Kumar Sharma, Mohamed A. El-Sheikh, Sheikh Mansoor.

Funding acquisition: Mohamed A. El-Sheikh, Shaista Qadir, Sheikh Mansoor.

Investigation: Ashutosh Srivastava, Shaista Qadir.

Methodology: Ashutosh Srivastava.

Project administration: Prashant Kaushik.

Resources: Vijay Kumar Sharma.

Software: Vijay Kumar Sharma.

Supervision: Prashant Kaushik.

Validation: Prashant Kaushik.

Visualization: Vijay Kumar Sharma, Prashant Kaushik.

Writing – original draft: Vijay Kumar Sharma, Prashant Kaushik.

Writing – review & editing: Prashant Kaushik, Mohamed A. El-Sheikh, Shaista Qadir, Sheikh Mansoor.

References

1. Ahmad P, Sarwat M, Bhat NA, Wani MR, Kazi AG, Tran L-SP. Alleviation of cadmium toxicity in *Brassica juncea* L.(Czern. & Coss.) by calcium application involves various physiological and biochemical strategies. PloS one. 2015; 10(1):e0114571. https://doi.org/10.1371/journal.pone.0114571 PMID: 25629695

2. Font R, Del Río M, Fernández-Martínez JM, de Haro-Bailón A. Use of near-infrared spectroscopy for screening the individual and total glucosinolate contents in Indian mustard seed (*Brassica juncea* L. Czern. & Coss.). Journal of agricultural and food chemistry. 2004; 52(11):3563–9. https://doi.org/10.1021/jf0307649 PMID: 15161231

3. Kopsell DA, McElroy JS, Sams CE, Kopsell DE. Genetic variation in carotenoid concentrations among diploid and amphidiploid rapid-cycling Brassica species. HortScience. 2007; 42(3):461–5.

4. Ge XT, Li YP, Wan ZJ, You MP, Finnegan PM, Banga SS, et al. Delineation of Sclerotinia sclerotiorum pathotypes using differential resistance responses on *Brassica napus* and *B. juncea* genotypes enables identification of resistance to prevailing pathotypes. Field Crops Research. 2012; 127:248–58.

5. Oram R, Kirk J, Veness P, Hurlstone C, Edlington J, Halsall D. Breeding Indian mustard [*Brassica juncea* (L.) Czern.] for cold-pressed, edible oil production—a review. Australian Journal of Agricultural Research. 2005; 56(6):581–96.

6. Begum N, Ahanger MA, Su Y, Lei Y, Mustafa NSA, Ahmad P, et al. Improved drought tolerance by AMF inoculation in maize (*Zea mays*) involves physiological and biochemical implications. Plants. 2019; 8 (12):579. https://doi.org/10.3390/plants8120579 PMID: 31817760

7. Kaur J, Akhatar J, Goyal A, Kaur N, Kaur S, Mittal M, et al. Genome wide association mapping and candidate gene analysis for pod shatter resistance in *Brassica juncea* and its progenitor species. Molecular biology reports. 2020:1–12. https://doi.org/10.1007/s11033-019-04608-x PMID: 31813129

8. Hasanuzzaman M, Hossain MA, da Silva JAT, Fujita M. Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. Crop stress and its management: perspectives and strategies: Springer; 2012. p. 261–315.

9. Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Critical reviews in biotechnology. 2010; 30(3):161–75. https://doi.org/10.3109/07388550903524243 PMID: 20214435

10. Ahmad P, Ahanger MA, Alyemeni MN, Wijaya L, Egamberdieva D, Bhardwaj R, et al. Zinc application mitigates the adverse effects of NaCl stress on mustard [*Brassica juncea* (L.) Czern & Coss] through modulating compatible organic solutes, antioxidant enzymes, and flavonoid content. Journal of Plant Interactions. 2017; 12(1):429–37.

11. de Oliveira AB, Alencar NLM, Gomes-Filho E. Comparison between the water and salt stress effects on plant growth and development. Responses of organisms to water stress. 2013; 67.

12. Lisar S, Motafakkerazad R, Hossain MM, Rahman I. Causes, Effects and Responses. Water stress. 2012; 25(1).

13. Kaur H, Sirhindi G, Bhardwaj R, Alyemeni M, Siddique KH, Ahmad P. 28-homobrassinolide regulates antioxidant enzyme activities and gene expression in response to salt-and temperature-induced oxidative stress in Brassica juncea. Scientific Reports. 2018; 8(1):1–13. https://doi.org/10.1038/s41598-017-17765-5 PMID: 29311619

14. McKersie BD, Lesheim Y. Stress and stress coping in cultivated plants: Springer Science & Business Media; 2013. https://doi.org/10.1111/pbi.12104 PMID: 23941360

15. Alferez F, Alquezar B, Burns JK, Zacarias L. Variation in water, osmotic and turgor potential in peel of 'Marsh'grapefruit during development of postharvest peel pitting. Postharvest Biology and Technology. 2010; 56(1):44–9.

16. Kohli SK, Khanna K, Bhardwaj R, Abd_Allah EF, Ahmad P, Corpas FJ. Assessment of subcellular ROS and NO metabolism in higher plants: multifunctional signaling molecules. Antioxidants. 2019; 8 (12):641. https://doi.org/10.3390/antiox8120641 PMID: 31842380

17. Qureshi MK, Munir S, Shahzad AN, Rasul S, Nouman W, Aslam K. Role of reactive oxygen species and contribution of new players in defense mechanism under drought stress in rice. Int J Agric Biol. 2018; 20(6):1339–52.

18. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of botany. 2012; 2012.

19. Ali S, Rizwan M, Hussain A, ur Rehman MZ, Ali B, Yousaf B, et al. Silicon nanoparticles enhanced the growth and reduced the cadmium accumulation in grains of wheat (Triticum aestivum L.). Plant Physiology and Biochemistry. 2019; 140:1–8. https://doi.org/10.1016/j.plaphy.2019.04.041 PMID: 31078051

20. Coskun D, Britto DT, Huynh WQ, Kronzucker HJ. The role of silicon in higher plants under salinity and drought stress. Frontiers in plant science. 2016; 7:1072. https://doi.org/10.3389/fpls.2016.01072 PMID: 27486474

21. Etesami H, Jeong BR. Silicon (Si): Review and future prospects on the action mechanisms in alleviating biotic and abiotic stresses in plants. Ecotoxicology and environmental safety. 2018; 147:881–96. https://doi.org/10.1016/j.ecoenv.2017.09.063 PMID: 28968941

22. Zhang W, Xie Z, Lang D, Cui J, Zhang X. Beneficial effects of silicon on abiotic stress tolerance in legumes. Journal of Plant Nutrition. 2017; 40(15):2224–36.

23. Guntzer F, Keller C, Meunier J-D. Benefits of plant silicon for crops: a review. Agronomy for Sustainable Development. 2012; 32(1):201–13.

24. Hartley SE, Fitt RN, McLarnon EL, Wade RN. Defending the leaf surface: intra-and inter-specific differences in silicon deposition in grasses in response to damage and silicon supply. Frontiers in Plant Science. 2015; 6:35. https://doi.org/10.3389/fpls.2015.00035 PMID: 25717331

25. Tubaña BS, Heckman JR. Silicon in soils and plants. Silicon and plant diseases: Springer; 2015. p. 7–51.

26. Farooq MA, Dietz K-J. Silicon as versatile player in plant and human biology: overlooked and poorly understood. Frontiers in plant science. 2015; 6:994. https://doi.org/10.3389/fpls.2015.00994 PMID: 26617630

27. Zhu Y, Gong H. Beneficial effects of silicon on salt and drought tolerance in plants. Agronomy for sustainable development. 2014; 34(2):455–72.

28. Vishwakarma K, Upadhyay N, Kumar N, Yadav G, Singh J, Mishra RK, et al. Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. Frontiers in plant science. 2017; 8:161. https://doi.org/10.3389/fpls.2017.00161 PMID: 28265276

29. Ahmed M, Qadeer U, Aslam MA. Silicon application and drought tolerance mechanism of sorghum. African Journal of Agricultural Research. 2011; 6(3):594–607.

30. Karmollachaab A, Gharineh MH. Effect of silicon application on wheat seedlings growth under water-deficit stress induced by polyethylene glycol. Iran Agricultural Research. 2015; 34(1):31–8.

31. Meena V, Dotaniya M, Coumar V, Rajendiran S, Kundu S, Rao AS. A case for silicon fertilization to improve crop yields in tropical soils. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2014; 84(3):505–18.

32. Alzahrani Y, Kuşvuran A, Alharby HF, Kuşvuran S, Rady MM. The defensive role of silicon in wheat against stress conditions induced by drought, salinity or cadmium. Ecotoxicology and environmental safety. 2018; 154:187–96. https://doi.org/10.1016/j.ecoenv.2018.02.057 PMID: 29475124

33. Gong H, Chen K. The regulatory role of silicon on water relations, photosynthetic gas exchange, and carboxylation activities of wheat leaves in field drought conditions. Acta Physiologiae Plantarum. 2012; 34(4):1589–94.

34. Kaushik P, Saini DK. Silicon as a vegetable crops modulator—A review. Plants. 2019; 8(6):148. https://doi.org/10.3390/plants8060148 PMID: 31159270

35. Chen M, Yang G, Sheng Y, Li P, Qiu H, Zhou X, et al. Glomus mosseae inoculation improves the root system architecture, photosynthetic efficiency and flavonoids accumulation of liquorice under nutrient stress. Frontiers in plant science. 2017; 8:931. https://doi.org/10.3389/fpls.2017.00931 PMID: 28638391

36. Panneerselvam P, Mohandas S, Saritha B, Upreti KK, Poovarasan Monnappa A, et al. Glomus mosseae associated bacteria and their influence on stimulation of mycorrhizal colonization, sporulation, and growth promotion in guava (*Psidium guajava* L.) seedlings. Biological Agriculture & Horticulture. 2012; 28(4):267–79.

37. Garg N, Bhandari P. Silicon nutrition and mycorrhizal inoculations improve growth, nutrient status, K +/Na+ ratio and yield of Cicer arietinum L. genotypes under salinity stress. Plant Growth Regulation. 2016; 78(3):371–87.

38. Ruiz-Lozano JM, Porcel R, Azcón C, Aroca R. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. Journal of Experimental Botany. 2012; 63(11):4033–44. https://doi.org/10.1093/jxb/ers126 PMID: 22553287

39. He C, Ma J, Wang L. A hemicellulose-bound form of silicon with potential to improve the mechanical properties and regeneration of the cell wall of rice. New Phytologist. 2015; 206(3):1051–62. https://doi.org/10.1111/nph.13282 PMID: 25615017

40. Yamamoto T, Nakamura A, Iwai H, Ishii T, Ma JF, Yokoyama R, et al. Effect of silicon deficiency on secondary cell wall synthesis in rice leaf. Journal of Plant Research. 2012; 125(6):771–9. https://doi.org/10.1007/s10265-012-0489-3 PMID: 22527842

41. Fauteux F, Rémus-Borel W, Menzies JG, Bélanger RR. Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiology letters. 2005; 249(1):1–6. https://doi.org/10.1016/j.femsle.2005.06.034 PMID: 16006059

42. Ahmad P, Ahanger MA, Alam P, Alyemeni MN, Wijaya L, Ali S, et al. Silicon (Si) supplementation alleviates NaCl toxicity in mung bean [*Vigna radiata* (L.) Wilczek] through the modifications of physio-biochemical attributes and key antioxidant enzymes. Journal of Plant Growth Regulation. 2019; 38(1):70–82.

43. Malhi GS, Kaur M, Kaushik P, Alyemeni MN, Alsahli AA, Ahmad P. Arbuscular mycorrhiza in combating abiotic stresses in vegetables: An eco-friendly approach. Saudi Journal of Biological Sciences. 2021; 28(2):1465. https://doi.org/10.1016/j.sjbs.2020.12.001 PMID: 33613074

44. Rizwan M, Ali S, ur Rehman MZ, Malik S, Adrees M, Qayyum MF, et al. Effect of foliar applications of silicon and titanium dioxide nanoparticles on growth, oxidative stress, and cadmium accumulation by rice (*Oryza sativa*). Acta physiologiae plantarum. 2019; 41(3):1–12.

45. Sakurai G, Satake A, Yamaji N, Mitani-Ueno N, Yokozawa M, Feugier FG, et al. In silico simulation modeling reveals the importance of the Casparian strip for efficient silicon uptake in rice roots. Plant and Cell Physiology. 2015; 56(4):631–9. https://doi.org/10.1093/pcp/pcv017 PMID: 25673476

46. Hinrichs M, Fleck AT, Biedermann E, Ngo NS, Schreiber L, Schenk MK. An ABC transporter is involved in the silicon-induced formation of casparian bands in the exodermis of rice. Frontiers in Plant Science. 2017; 8:671. https://doi.org/10.3389/fpls.2017.00671 PMID: 28503184

47. Horie T, Karahara I, Katsuhara M. Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. Rice. 2012; 5(1):1–18. https://doi.org/10.1186/1939-8433-5-1 PMID: 24764501

48. Thorne SJ, Hartley SE, Maathuis FJ. Is silicon a panacea for alleviating drought and salt stress in crops? Frontiers in plant science. 2020; 11:1221. https://doi.org/10.3389/fpls.2020.01221 PMID: 32973824

49. Pommerrenig B, Diehn TA, Bienert GP. Metalloido-porins: Essentiality of Nodulin 26-like intrinsic proteins in metalloid transport. Plant Science. 2015; 238:212–27. https://doi.org/10.1016/j.plantsci.2015.06.002 PMID: 26259189

50. Manivannan A, Ahn Y-K. Silicon regulates potential genes involved in major physiological processes in plants to combat stress. Frontiers in Plant Science. 2017; 8:1346. https://doi.org/10.3389/fpls.2017.01346 PMID: 28824681

51. Dionisio-Sese ML, Tobita S. Antioxidant responses of rice seedlings to salinity stress. Plant science. 1998; 135(1):1–9.

52. Bijalwan P, Jeddi K, Saini I, Sharma M, Kaushik P, Hessini K. Mitigation of saline conditions in watermelon with mycorrhiza and silicon application. Saudi Journal of Biological Sciences. 2021. https://doi.org/10.1016/j.sjbs.2021.05.019 PMID: 34220218

53. Sarwat M, Hashem A, Ahanger MA, Abd_Allah EF, Alqarawi A, Alyemeni MN, et al. Mitigation of NaCl stress by arbuscular mycorrhizal fungi through the modulation of osmolytes, antioxidants and secondary metabolites in mustard (*Brassica juncea* L.) plants. Frontiers in plant science. 2016; 7. https://doi.org/10.3389/fpls.2016.00869 PMID: 27458462

54. Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, et al. Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Frontiers in plant science. 2019; 10:1068. https://doi.org/10.3389/fpls.2019.01068 PMID: 31608075

55. Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, et al. Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Scientia Horticulturae. 2015; 196:91–108.

56. Liu H, Wang Y, Hart M, Chen H, Tang M. Arbuscular mycorrhizal symbiosis regulates hormone and osmotic equilibrium of Lycium barbarum L. under salt stress. Mycosphere. 2016; 7(6):828–43.

57. Yasmeen T, Tariq M, Iqbal S, Arif MS, Riaz M, Shahzad SM, et al. Ameliorative capability of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) against salt stress in plant. Plant Abiotic Stress Tolerance. 2019:409–48.

58. Lin J, Wang Y, Sun S, Mu C, Yan X. Effects of arbuscular mycorrhizal fungi on the growth, photosynthesis and photosynthetic pigments of Leymus chinensis seedlings under salt-alkali stress and nitrogen deposition. Science of the Total Environment. 2017; 576:234–41.

59. Yadav A, Saini I, Kaushik P, Ansari MA, Khan MR, Haq N. Effects of arbuscular mycorrhizal fungi and P-solubilizing *Pseudomonas fluorescence* (ATCC-17400) on morphological traits and mineral content of sesame. Saudi Journal of Biological Sciences. 2021; 28(5):2649–54. https://doi.org/10.1016/j.sjbs.2021.03.024 PMID: 34025149

60. Ahanger MA, Bhat JA, Siddiqui MH, Rinklebe J, Ahmad P. Integration of silicon and secondary metabolites in plants: a significant association in stress tolerance. Journal of experimental botany. 2020; 71 (21):6758–74. https://doi.org/10.1093/jxb/eraa291 PMID: 32585681

61. Malik MA, Wani AH, Mir SH, Rehman IU, Tahir I, Ahmad P, et al. Elucidating the role of silicon in drought stress tolerance in plants. Plant Physiology and Biochemistry. 2021; 165:187–95. https://doi.org/10.1016/j.plaphy.2021.04.021 PMID: 34049031

62. Moradtalab N, Hajiboland R, Aliasgharzad N, Hartmann TE, Neumann G. Silicon and the association with an arbuscular-mycorrhizal fungus (*Rhizophagus clarus*) mitigate the adverse effects of drought stress on strawberry. Agronomy. 2019; 9(1):41.

63. Shi Y, Zhang Y, Yao H, Wu J, Sun H, Gong H. Silicon improves seed germination and alleviates oxidative stress of bud seedlings in tomato under water deficit stress. Plant Physiology and Biochemistry. 2014; 78:27–36. https://doi.org/10.1016/j.plaphy.2014.02.009 PMID: 24607576

64. Jan S, Alyemeni MN, Wijaya L, Alam P, Siddique KH, Ahmad P. Interactive effect of 24-epibrassinolide and silicon alleviates cadmium stress via the modulation of antioxidant defense and glyoxalase systems and macronutrient content in Pisum sativum L. seedlings. BMC plant biology. 2018; 18(1):1–18. https://doi.org/10.1186/s12870-017-1213-1 PMID: 29291729

65. Atakan A, Özkaya HÖ, Erdoğan O. Effects of arbuscular mycorrhizal fungi (AMF) on heavy metal and salt stress. Turkish Journal of Agriculture-Food Science and Technology. 2018; 6(11):1569–74.

66. Kaushik P, Sandhu OS, Brar NS, Kumar V, Malhi GS, Kesh H, et al. Soil Metagenomics: Prospects and Challenges. Mycorrhizal Fungi-Utilization in Agriculture and Industry: IntechOpen; 2020.

67. Pal A, Pandey S. Role of glomalin in improving soil fertility. International journal of plant and soil science. 2014; 3:112–29.

68. Goyal D, Mishra S, Dantu PK. Arbuscular Mycorrhizae Associations and Role in Mitigation of Drought Stress in Plants. Agriculturally Important Fungi for Sustainable Agriculture: Springer; 2020. p. 81–100.

69. Mathimaran N, Sharma MP, Mohan Raju B, Bagyaraj D. Arbuscular mycorrhizal symbiosis and drought tolerance in crop plants. Mycosphere. 2017; 8(3):361–76.

70. Chakraborty U, Chakraborty B, Dey P, Chakraborty AP. Role of microorganisms in alleviation of abiotic stresses for sustainable agriculture. Abiotic stresses in crop plants. 2015:232–53.

71. Arif Y, Singh P, Siddiqui H, Bajguz A, Hayat S. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiology and Biochemistry. 2020; 156:64–77. https://doi.org/10.1016/j.plaphy.2020.08.042 PMID: 32906023

72. Kapoor R, Singh N. Arbuscular mycorrhiza and reactive oxygen species. Arbuscular mycorrhizas and stress tolerance of plants: Springer; 2017. p. 225–43.

73. Quiroga G, Erice G, Aroca R, Zamarreño ÁM, García-Mina JM, Ruiz-Lozano JM. Arbuscular mycorrhizal symbiosis and salicylic acid regulate aquaporins and root hydraulic properties in maize plants subjected to drought. Agricultural water management. 2018; 202:271–84.

74. Copetta A, Bardi L, Bertolone E, Berta G. Fruit production and quality of tomato plants (*Solanum lycopersicum* L.) are affected by green compost and arbuscular mycorrhizal fungi. Plant biosystems. 2011; 145(1):106–15.

75. Wu Q-S, Zou Y-N. Arbuscular mycorrhizal fungi and tolerance of drought stress in plants. Arbuscular mycorrhizas and stress tolerance of plants: Springer; 2017. p. 25–41.

76. Giovannetti M, Avio L, Sbrana C. Functional significance of anastomosis in arbuscular mycorrhizal networks. Mycorrhizal networks: Springer; 2015. p. 41–67.

77. Novais CBd, Pepe A, Siqueira JO, Giovannetti M, Sbrana C. Compatibility and incompatibility in hyphal anastomosis of arbuscular mycorrhizal fungi. Scientia Agricola. 2017; 74:411–6.

78. Sahebi M, Hanafi MM, Siti Nor Akmar A, Rafii MY, Azizi P, Tengoua F, et al. Importance of silicon and mechanisms of biosilica formation in plants. BioMed research international. 2015; 2015. https://doi.org/10.1155/2015/396010 PMID: 25685787

79. Soukup M, Rodriguez Zancajo VM, Kneipp J, Elbaum R. Formation of root silica aggregates in sorghum is an active process of the endodermis. Journal of experimental botany. 2020; 71(21):6807–17. https://doi.org/10.1093/jxb/erz387 PMID: 31504726

80. Ahmed M, Kamran A, Asif M, Qadeer U, Ahmed ZI, Goyal A. Silicon priming: a potential source to impart abiotic stress tolerance in wheat: A review. Australian Journal of Crop Science. 2013; 7(4):484–91.

81. Waraich EA, Ahmad R, Ashraf M. Role of mineral nutrition in alleviation of drought stress in plants. Australian Journal of Crop Science. 2011; 5(6):764–77.

82. Zhang Y, Liang Y, Zhao X, Jin X, Hou L, Shi Y, et al. Silicon compensates phosphorus deficit-induced growth inhibition by improving photosynthetic capacity, antioxidant potential, and nutrient homeostasis in tomato. Agronomy. 2019; 9(11):733.

83. Vahdati K, Lotfi N. Abiotic stress tolerance in plants with emphasizing on drought and salinity stresses in walnut. Abiotic Stress–Plant Responses and Applications in Agriculture. 2013; 10:307–65.

84. Iqbal N, Umar S, Khan NA. Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). Journal of Plant Physiology. 2015; 178:84–91. https://doi.org/10.1016/j.jplph.2015.02.006 PMID: 25800225

85. Singh A, Sharma MK, Sengar R. Osmolytes: Proline metabolism in plants as sensors of abiotic stress. Journal of Applied and Natural Science. 2017; 9(4):2079–92.

86. Anwar Hossain M, Golam Mostof M, Fujita M. Modulation of reactive oxygen species and methylglyoxal detoxification systems by exogenous glycinebetaine and proline improves drought tolerance in mustard (*Brassica juncea* L.). International Journal of Plant Biology & Research. 2014.

87. Rady MM, Elrys AS, El-Maati MFA, Desoky E-SM. Interplaying roles of silicon and proline effectively improve salt and cadmium stress tolerance in Phaseolus vulgaris plant. Plant Physiology and Biochemistry. 2019; 139:558–68. https://doi.org/10.1016/j.plaphy.2019.04.025 PMID: 31029029

88. Mauad M, Crusciol CAC, Nascente AS, Grassi H, Lima GPP. Effects of silicon and drought stress on biochemical characteristics of leaves of upland rice cultivars. Revista Ciência Agronômica. 2016; 47:532–9.

89. Raza MAS, Haider I, Farrukh Saleem M, Iqbal R, Usman Aslam M, Ahmad S, et al. Integrating biochar, rhizobacteria and silicon for strenuous productivity of drought stressed wheat. Communications in Soil Science and Plant Analysis. 2021; 52(4):338–52.

90. Baslam M, Goicoechea N. Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. Mycorrhiza. 2012; 22(5):347–59. https://doi.org/10.1007/s00572-011-0408-9 PMID: 21894519

91. Shahid M, Pourrut B, Dumat C, Nadeem M, Aslam M, Pinelli E. Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. Reviews of Environmental Contamination and Toxicology Volume 232. 2014:1–44. https://doi.org/10.1007/978-3-319-06746-9_1 PMID: 24984833

92. Nadeem SM, Ahmad M, Zahir ZA, Javaid A, Ashraf M. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnology advances. 2014; 32(2):429–48. https://doi.org/10.1016/j.biotechadv.2013.12.005 PMID: 24380797

93. Vilela LA, Teixeira AF, Lourenço FM, Souza MD. Symbiotic microorganisms enhance antioxidant defense in plants exposed to metal/metalloid-contaminated soils. Plants Under Metal and Metalloid Stress: Springer; 2018. p. 337–66.

94. Howladar SM, Al-Robai SA, Al-Zahrani FS, Howladar MM, Aldhebiani AY. Silicon and its application method effects on modulation of cadmium stress responses in *Triticum aestivum* (L.) through improving the antioxidative defense system and polyamine gene expression. Ecotoxicology and environmental safety. 2018; 159:143–52. https://doi.org/10.1016/j.ecoenv.2018.05.004 PMID: 29738930

95. Farooq MA, Ali S, Hameed A, Ishaque W, Mahmood K, Iqbal Z. Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. Ecotoxicology and environmental safety. 2013; 96:242–9. https://doi.org/10.1016/j.ecoenv.2013.07.006 PMID: 23911213