FOLIAGE APPLIED SILICON AMELIORATES DROUGHT STRESS THROUGH PHYSIO-MORPHOLOGICAL TRAITS, OSMOPROTECTANTS AND ANTIOXIDANT METABOLISM OF CAMELINA (Camelina sativa L.) GENOTYPES

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ABSTRACT

Silicon (Si) is one of the best plant defense elements against the biotic and abiotic stresses. Camelina plants accumulate Si which serves in protection against drought stress. The present study was conducted to investigate the impact of different doses of foliage applied Si (0, 3, 6 and 9 mM) under water stress (40% field capacity, FC) and non-stress conditions (100% FC) on camelina genotypes (Canadian and Australian). The imposed drought drastically decreased the growth parameters like root-shoot length and plant fresh and dry weight and also had negative impact on the chlorophyll content along with water relation attributes (water potential, osmotic potential and turgor pressure). In contrast, total free amino acids, total soluble proteins, proline and antioxidants such as ascorbic peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were enhanced especially in water stressed Canadian genotype, while osmoprotectants (flavonoids, anthocyanins and glycinebetaine) and phenolics contents were decreased. On the other hand, the foliar application of Si was instrumental in enhancing the growth of camelina by increasing the chlorophyll contents and water relation of stressed and non-stressed plants. Similarly, the biochemical, osmoprotectants and antioxidant metabolism was also improved in camelina stressed plants through the application of foliar Si. In conclusion, foliar application of 6 mM Si at vegetative growth stage played a vital role in alleviating the drastic impact of water stress on camelina growth by improving the water status, chlorophyll content, accumulation of phenolics and osmoprotectants and activating antioxidants. Therefore, the foliar application of Si could be developed as an important biologically viable strategy for boosting the tolerance in camelina plants to water stress conditions.

Key words: antioxidants, biochemistry, camelina, drought stress, osmoprotectants, physiology and silicon
INTRODUCTION

Climate change is one of the most intricate challenges faced by sustainable crop production in the 21st century. The seasonal and unseasonal variations in temperature and precipitation have made the drought stress a major limiting factor for the agricultural productivity [EL Sabagh et al. 2019, Iqbal 2019, Faisal et al. 2020]. Drought is an important threat and a key abiotic stress factor that limits the crops growth and yield irrelevant of occurrence at any stage of the crop [Ahmad et al. 2017]. Water stress was reported to diminish tissue water potential, photosynthesis rate, nitrogen metabolism, protein synthesis and cell membrane stability in plants which resulted in decreased final crop yield [Saneoka et al. 2004, Islam et al. 2019, Bazzaz et al. 2020]. Plants cope with water stress conditions through physiological adaptations which help plants to withstand stresses during their life cycle [Reddy et al. 2003]. To meet the emerging challenge of frequent and severe drought stress, the application of stress-tolerant cultivars may be the good economic and biological solution [EL Sabagh et al. 2020, Zahoor et al. 2020].

Silicon (Si) enhances the metabolic, physiological and structural stability in plants [Bukhari et al. 2015]. The ameliorative effects of Si under water stress – are well documented in rice [Ming et al. 2012], maize [Kaya et al. 2006] and wheat [Pei et al. 2010]. Although, in case of growth of plants, the Si is the non-essential nutrient, scientists reported that the foliar application of Si showed the better growth of plants in control as well as water stressful environment [Ahmed et al. 2011, Bukhari et al. 2015, Iqbal et al. 2017, Iqbal et al. 2019]. There have been few reports which discussed the role and mechanism of Si in alleviating drought stress in plants by accumulating compatible solutes, activation of enzymes and physiological attributes in camelina.

Camelina (Camelina sativa L.) belongs to Brassicaceae family and is an alternative annual oilseed crop [Gesch 2014]. It is fast growing, an annual spring-planted crop with 80–100 days of its life cycle. These plants can grow up to 1.0 m height and are pre-dominantly the self-pollinated crop. Pods of camelina are in teardrop shaped having 12–18 seeds per pod with 5–6 mm in diameter. Seeds are very small even when compared with mustard family [Gugel and Falk 2006]. Camelina plant is considered to be more biotic and abiotic stress tolerant than canola and other grain legume crops [Schillinger et al. 2012, Wysociki et al. 2013]. The camelina oil seed crop becomes is used in bio-products as well as in biofuels [Gesch 2014], as diesel and jet fuel [Moser 2008]. Oil of camelina seeds is popular and healthy as cooking oil for humans [Pilgeram et al. 2007, Lu and Kang 2008]. It is rich in omega-3 fatty acids due to the presence of linolenic acid [Hrastar et al. 2009] and has improved oxidative stability [Abramovic and Abram 2005]. Being a nitrogen (N) efficient crop, camelina need low quantity of N for the production of higher yields than canola [Lenssen et al. 2012, Wysocki et al. 2013]. Under water deficit, the growth and productivity of these crops are decreased [Waraich et al. 2020a, Waraich et al. 2020b].

As camelina is new oil seed crop in Pakistan and there are inconclusive and varying results with reference to Camelina sativa production in different areas of the world, thus this study was designed to investigate the response of different camelina to foliar application of Si under water deficit conditions. To date, the literature about the role of foliar application of Si to mediate drought stress especially on camelina oil seed crop has not yet been available. Therefore, we designed an experiment to check the effect of foliar application of Si on growth, physiology, biochemistry, osmoprotectants content and antioxidant enzyme activities of camelina growing under normal and water stress conditions.

MATERIALS AND METHODS

Study area and experimental procedure. Current research work was implemented at the Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, Punjab, Pakistan. The camelina genotypes (Australian camelina and Canadian camelina) were grown under natural conditions during the growing season of 2016. The sterilized seeds of camelina genotypes were sown with three replications in pots (five seed per pot) filled with 500 g of dried sand under completely randomized design with factorial arrangement. The pots were irrigated according to their field capacity after sowing of both genotypes of camelina. At the time of sowing of seed the solution of different
nutrients as a basal were applied to the soil (Pre-Plant) in ml/kg soil: ammonium nitrate (NH₄NO₃), 1.67; monopotassium phosphate (KH₂PO₄), 1.67; potassium sulfate (K₂SO₄), 3.33; CaCl₂·2H₂O, 1.67; NH₄NO₃, 1.67; magnesium sulfate (MgSO₄·7H₂O), 3.33; magnesium(II) sulfate monohydrate (MnSO₄·H₂O), 1.67; zinc sulfate heptahydrate (ZnSO₄·7H₂O), 1.67; copper(II) sulfate pentahydrate (CuSO₄·5H₂O), 1.67; boric acid (H₃BO₃), 1.67; cobalt(II) sulfate heptahydrate (CoSO₄·7H₂O), 1.67; sodium molybdate (Na₂MoO₄·2H₂O), 1.67. All pots were irrigated at normal field capacity up to the complete germination of seeds. At complete germination the pots were divided into two groups, one group under water stress conditions (40% field capacity, FC) and other group under normal conditions (100% FC). After 25 days of sowing, four doses of Si were foliar applied (control, 3 mM, 6 mM and 9 mM Si) using sodium silicate (Na₂SiO₃) as a source of Si. Both genotypes of camelina were harvested after 40 days of sowing for further analysis and data on following plant traits were recorded.

**Growth characters.** Growth characters such as root and shoot length and their fresh and dry weights were measured after harvesting the camelina crop.

**Water relations attribute.** Scholander type pressure chamber was used for the measurement of leaf water potential by selecting the top leaf of plant which was fully expanded. After recording the leaf water potential, the leaf was frozen at -20°C for the further measurements of osmotic potential using method by Nawaz et al. [2015]. Turgor potential was calculated as the difference between osmotic potential (ψs) and water potential (ψw) values (ψp = ψw – ψs).

**Measurement of chlorophyll content.** The total chlorophyll content of the leaf was measured with SPAD-502 using the method of Ehsanzadeh et al. [2009].

**Measurement of biochemical parameters.** The total soluble sugars (TSS) and total soluble proteins (TSP) were determined using the method of Irigoyen et al. [1992] and Bradford [1976], respectively. Contents of total free amino acids (TFAA) and proline were measured following Lee and Takanashi [1966] and Bates et al. [1973], respectively.

**Measurements of osmoprotectants and total phenolics contents.** Anthocyanins were extracted from the oven-dried ground tissues by suspending in 10 ml of acidified methanol (methanol: water: HCl, 79 : 20 : 1, v/v) and auto extracting at 0°C for 72 hours in dark with continuous shaking. The extracts were then centrifuged for 10 min at 5000 rpm and the absorbance was measured at 530 and 657 nm. The absorbance at 530 nm (A530) was corrected for scattering using the absorbance at 657 nm (A657) according to Rayleigh’s formula: Corrected absorbance A530 = A530 – 1/3 A657 [Lange et al. 1971]. The flavonoid content was calculated by the method described by Ordonéz et al. [2006]. To 0.5 mL of sample 0.5 mL of AlCl₃ solution (2% in absolute ethanol) was added. After one hour of incubation at temperature room, the absorbance was measured at 420 nm, and then the total flavonoid content was calculated in terms of quercetin as a reference to the standard curve.

Glycinebetaine content was measured in camelina samples following Grieve and Gratan [1983]. Fresh leaf material (1.0 g) was homogenized in 10 mL of distilled water. 1 mL of filtrated extract was acidified by adding 1 mL of hydrochloric acid (2N) and 0.5 mL of this acidified solution was taken in test tubes having 0.2 mL of potassium tri-iodide solution. This reaction mixture was placed on random shaking in ice bath for 90 min. After shaking, 2 mL of chilled distilled water and 20 mL of 1, 2 dichloroethane (cooled at -10°C) were added to the mixture. Samples were vortex to pass a continuous stream of air for 1–2 min to blend the double layered solution. The redundant upper aqueous layer was discarded and absorbance for organic layer was read at 365 nm using spectrophotometer (Hitachi-150-20, Japan). The standard curve was used to compute the concentrations of the glycinebetaine.

The method of Waterhouse [2001] was used for the determination of soluble phenolics in total form. Leaf of 1 g was homogenized using 80% acetone (10 mL), then centrifuged at 400 rpm for 10 min. After centrifugation, 20 μL of extract was taken in a test tube along with 1.58 mL water, 100 μL of Folin-Ciocalteu reagent, to which 300 μL of sodium carbonate solution was added and kept at 40°C for 30 min. Absorbance of the samples was measured at 765 nm.

**Assay of antioxidant enzyme activities.** The activities of different antioxidants such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and...
A

Shoot length (cm)

B

Shoot fresh weight (g)

C

Shoot dry weight (g)

D

Root length (cm)
Fig. 1. (A-F): Effect of foliar application of silicon (Si) on growth parameters of two camelina genotypes in well-watered (100% FC) and water stress (40% FC) conditions (mean values ±S.E.)

ascorbate peroxidase (APX) were measured spectrophotometrically using 1 g of fresh leaf sample which was homogenized in 50 mM phosphate buffer of pH 7.0 and 1 mM DTT [Dixit et al. 2001]. The CAT and POD activities were measured following Zhang et al. [2012], while the SOD and APX – were determined by the methods given by Cakmak [1994] and Giannopilitis and Ries [1997].

**Statistical analysis.** Data were analyzed statistically using analysis of variance (ANOVA) with MSTAT-C. The significant differences among all mean values were measured using LSD test with 5% probability level.

**RESULTS**

**Growth parameters.** Water stress negatively influenced the growth of camelina, while the foliar application of Si improved plant growth compared to the control conditions (Tab. 1). The foliar application of 6 mM Si at the vegetative stage of camelina improved the growth parameters such as root-shoot length and their fresh and dry weights under both water stress (40% FC) and non-stress conditions (100% FC) as compared to all other treatments shown in Figure 1A-F.

**Water relation parameters and total chlorophyll content.** The water stress conditions declined the total Chl content and the water relation parameters in camelina leaf, while the foliar application of Si enhanced the total Chl content and water relation parameters under stress and non-stress conditions in both genotypes of camelina (Tab. 1). The different doses of applied Si presented positive response with reference to improvement in Chl content and water relations of camelina genotypes under both conditions. The 6 mM Si

**Table 1.** Analysis of variance of growth, water relations, biochemical antioxidant and osmoprotectants content of camelina under foliar application of Si in drought stress

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<th>Traits</th>
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<td>Glycinebetaine (µmol/g FW)</td>
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*, **, *** significant at 0.05, 0.01 and 0.001 level, respectively; NS non significant
Fig. 2. Effect of foliar applied silicon (Si) on total chlorophyll content (A), water potential (B), osmotic potential (C) and turgor pressure (D) of two camelina genotypes in well-watered (100% FC) and water stress (40% FC) conditions (mean values ±S.E.)
Fig. 3. Effect of foliar applied silicon (Si) on proline (A), TSS (B), TFAA (C) and TSP (D) of two camelina genotypes in well-watered (100% FC) and water stress (40% FC) conditions (mean values ± S.E.)
Effect of foliar application of silicon (Si) on content of total phenolics (A), anthocyanins (B), flavonoids (C) and glycinebetaine (D) of two camelina genotypes in well-watered (100% FC) and water stress (40% FC) conditions (mean values ±S.E.)
Fig. 5. Effect of foliar applied silicon (Si) on APX (A), SOD (B), POD (C) and CAT (D) of two camelina genotypes in well-watered (100% FC) and water stress (40% FC) conditions (mean values ±S.E.)
treatment was more responsive in improving the total Chl content and water relation of camelina genotypes under both water stress (40% FC) and non-stress conditions (100% FC) as compared to all other treatments including the control (Fig. 2A-D).

**Biochemical parameters.** The water stress reduced the biochemical parameters such as proline, TSS, and TSP content, but enhanced FTAA content in both genotypes of camelina leaf, while the foliar application of Si significantly improved these parameters in both genotypes of camelina under water stress and non-stress conditions (Tab. 1). Foliage applied 6 mM Si at vegetative stage improved production of all biochemical parameters studied under both conditions as shown in Figure 3A-D. Moreover, 9 mM Si performed better than the lowest dose of Si in enhancing the biochemical parameters of camelina genotypes.

**Osmoprotectants and phenolics contents.** The application of Si significantly affected the osmoprotectants (anthocyanins, flavonoids and glycinebetaine content) and phenolics content in camelina plants (both genotypes) under water stress and non-stress conditions (Tab. 1). The stress conditions decreased the anthocyanins, flavonoids and phenolics content in both genotypes of camelina leaf, and elevated glycinebetaine content. Moreover, content of the osmoprotectants and phenolics improved with the application of Si and was the highest after the application of 6 mM Si in comparison with all other treatments, including control. The minimum concentration of osmoprotectants and phenolics contents were noticed in control treatment where no Si was applied (Figure 4A-D).

**Antioxidants.** The antioxidant activities such as APX, SOD, POD and CAT in camelina genotypes were also significantly influenced by application of various doses of Si (Tab. 1). The activities of antioxidants were enhanced in water stress condition as compared to control. Highest increment was observed in 40% FC, while the minimum activities of these antioxidants were recorded in unstressed conditions (Fig. 5A-D). The maximum activity of antioxidants was noticed in water stress plants where 6 mM Si was applied. Similarly, the Si application also enhanced the antioxidant activities in non-stress conditions as compared to control.

**DISCUSSION**

The growth and physiology of all crops have been reported to be affected under water deficit conditions [Yao et al. 2009] while the application of Si enhanced the tolerance in plants under stressed environment [Kaya et al. 2006, Liang et al. 2008]. The current research showed that drought stress reduced all growth parameters of camelina, while the foliar application of 6 mM Si improved the growth of camelina under drought stress. Similar results were obtained by the Gong and Chen [2012] in wheat where Si application enhanced the growth of wheat under water stress. Chen et al. [2011] also reported that the application of Si was effective in improving tolerance in plants under abiotic stress conditions.

The application of Si improved the water relation parameters and total Chl content of camelina under water stress. These results are in accordance with the findings of Gong et al. [2003], who observed that the application of Si enhanced the leaf water content in wheat under water stress. The foliar application of Si increased the water potential in leaf due to deposition of silica in epidermis tissue of rice leaf [Matoh et al. 1991]. The turgor pressure in camelina genotypes was maintained by the application of foliar Si because the solutes accumulated in plant cells under stress conditions and water moved from the surroundings to the cell carrying out metabolic activities [Subbarao et al. 2000]. The drought tolerance in plants was improved by the application of Si and uptake of more water from the soil [Hattori et al. 2005 and 2007]. The application of Si was helpful in reduction of transcription losses due to deposition of silica on leaf and enhanced the total chlorophyll content in plants under water stress [Na and Jiashu 2001, Karmollachaab et al. 2014].

The osmoprotectants such as TSP, TSS and proline in camelina genotypes were reduced under water stress, while the foliar application of 6 mM Si improved these parameters under normal condition, but the TFAA increased under water stress. The proline content increases under water stress and showed a positive relationship between level of proline and water stress [Kumari and Sairam 2013] while the application of Si built negative relationship [Pei et al. 2010]. But in the present study, the foliar application of Si improved the accumulation of proline under stress condi-
tions. Under stress conditions, the light absorption to the leaf is lower which results in reduced TSS content in the leaves of plants [Hamdia and Shaddab 2010], while the application of Si enhanced the TSS content in camelina leaf under water stress. Our findings showed that TSP content in the leaf of camelina diverted synthesized protein for the growth into osmoregulation under stress conditions. The increase in TSP contents in plant by the application of Si reported by the Soundarajan et al. [2014] or either engaged active-ly in the formation of DNA and also in the functioning of mRNA [Abbas et al. 2015]. The present research depicted that the content of total phenolics, anthocya-nins, flavonoids and glycinebetaine of both genotypes of camelina were influenced under water stress, but the foliar application of 6 mM Si improved these parameters under stress conditions. To date, there is no literature available regarding the interaction of total phe-nolic contents, anthocyanin, flavonoids and glycinebe-taine and Si under water stress or normal conditions. The increase in total phenolic contents, anthocyanin, flavonoids and glycinebetaine in camelina leaf by the application of foliar Si may be due to the enhancement in chlorophyll fluorescence and photosynthesis in leaf of plants [Hattori et al. 2005].

The imbalance between the antioxidant activities and production of ROS due to the damage of photosynthetic pigments under drought stress conditions affects the crop productivity [Gong et al. 2005]. In the present research, the activities of antioxidants were improved by the application of Si under water stress condition in both genotypes of camelina and leading to drought tolerance. Similar result was also obtained by the Gong et al. [2005], who reported that drought tolerance was obtained due to the application of Si in wheat through the regulation of antioxidants activities. Shi et al. [2016] also reported that the supplemental application of Si enhanced tolerance in tomato plants by increasing the antioxidants activities along with improvement in water activity in roots under drought stress conditions.

CONCLUSION

The foliar application of silicon positively alleviated the drought stress by improving the growth characters, water relation parameters, osmoprotectants and antioxidant metabolism of oilseed camelina. Therefore, application of 6 mM Si under water stress conditions was more effective in alleviating the stress. Consequently, it was assumed that, the foliar application of Si may be developed as a significant biologically viable strategy for boosting the tolerance in camelina plants to water stress conditions.

ACKNOWLEDGEMENTS

The authors extend their appreciation to Taif University for funding current work by Taif University Researchers Supporting Project number (TURSP-2020/120), Taif University, Taif, Saudi Arabia.

FUNDING

The current work was funded by Taif University Researchers Supporting Project number (TURSP – 2020/120), Taif university, Taif, Saudi Arabia.

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