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Silicon modulates nitro-oxidative homeostasis along with the antioxidant metabolism to promote drought stress tolerance in lentil plants

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ABSTRACT

Lentil is the fifth most important grain legume growing in arid/semi-arid regions of the world. Drought is one of the major constraints leading up to 50 % of production losses just in lentil. Application of silicon (Si) has been shown to be a promising solution to improve drought tolerance; however, the biochemical mechanisms and interactions involved are not fully understood, especially in legumes. This study was designed to evaluate the effects of Si on drought stress tolerance of lentil genotypes. Seven lentil genotypes with different drought tolerance levels (tolerant, moderately tolerant and sensitive) were subjected to moderate and severe drought stress at the onset of the reproductive stage. Results showed that different drought stress treatments significantly decreased the above ground biomass, water status, and the concentration of chlorophyll pigments, whereas Si supplementation of drought stressed lentil genotypes significantly improved the same traits, irrespective of their drought tolerant levels. On the other hand, Si effect on osmoregulation lead to a decline in the membrane damage and osmolytes (proline and glycine betaine) concentration in drought-stressed lentil. Application of Si to drought stressed lentil plants, significantly maintained the nitro-oxidative homeostasis by balancing the concentrations of reactive oxygen/nitrogen species, superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and nitrous oxide (NO), thereby reducing the oxidative damage caused due to drought stress. Furthermore, Si supplementation also stimulated the efficiency of the glutathione-ascorbate cycle by increasing the concentrations of glutathione (GSH) and ascorbate (ASC) as well as the activities of antioxidant enzymes like ascorbate peroxidase (APX), guaiacol peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), dehydro-ascorbate reductase (DHAR) and nitrate reductase (NR) for better protection of cell membranes from reactive oxygen species. Although Si showed the same regulatory mechanisms in all the studied genotypes, to protect lentil plants from moderate and severe drought stress the defensive role of Si against drought stress was more conspicuous in drought sensitive genotypes than in the tolerant ones. Thus, this study suggests the protective role of Si on drought stressed lentil genotypes through the modulation of nitro-oxidative homeostasis and antioxidant defence responses.

Keywords: Antioxidant enzymes; Glutathione-ascorbate cycle; Osmolytes; Osmoregulation; Reactive oxygen species.

1. Introduction

Drought is a significant abiotic stress that influences the growth, physiological traits and related biochemical pathways of sensitive plants (Jones et al. 1981; Turner, 1986; Nilsen and Orcutt, 1996). Osmoregulation, or osmotic adjustment, is the main drought stress adaptive mechanism in plants (Blum, 2017; Turner, 2017; Perri et al. 2018). Plants accumulate osmolytes, such as proline (in cytosol and vacuole) and glycine betaine (GB) (in chloroplast), in response to drought stress to facilitate water uptake and protect cells against reactive oxygen species (ROS) accumulation (Turner and Jones, 1980; Morgan, 1984; Ashraf and Foolad, 2007). In plants, ROS such as superoxide radicals ($O_2^{\cdot-}$), hydroxyl radicals (.OH) and hydrogen peroxide (H_2O_2) are frequently produced as by-products of different metabolic pathways. The balance between production and removal of ROS in various cellular compartments (mitochondria, chloroplasts and peroxisomes) and the apoplast are maintained under physiological steady state conditions in plants (Apel and Hert, 2004). However, drought stress leads to a disequilibrium state by the over accumulation of ROS in cells. Similarly, the accumulation of reactive nitrogen species (RNS) such as nitrous oxide (NO) in peroxisomes and lipid peroxidation (LPX) in cell membranes under stressed environments can also cause irreversible damage to DNA, proteins, and lipids (Luis et al. 2006; Gill and Tuteja, 2010; Hasanuzzaman et al. 2012). Even though ROS and RNS are potential cytotoxic molecules, resulting in the generation of oxidative/nitrosative stress in plants, they also function as signal transduction molecules involved in growth regulation, development and defence responses, thus responsible for 'oxidative signalling' in plants (Miller et al. 2010; Luis et al. 2006; del Rio et al. 2015). ROS/RNS signalling during stress conditions is integrated into many of the other signalling cascades that regulate gene expression and subsequently, the plant adaptation (Apel and Hert, 2004; Luis et al. 2006). Metabolism of ROS and RNS must be integrated in plants because these two families of molecules are characterised by rigorous metabolic interplay in plants (Corpas and Barroso, 2013). To combat drought stress, plants are well equipped with a highly complex and dynamic network of ROS/RNS scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and nitrate reductase (NR), as well as antioxidants such as ascorbate (ASC) and glutathione (GSH) (Das and Roychoudhury, 2014). Thus, the antioxidant metabolism and homeostasis of ROS and RNS is critical for plant performance under drought stress conditions.

Silicon (Si), a tetravalent metalloid, is the second most abundant element after oxygen and is beneficial for plant growth (Ma and Takahashi, 2002; Epstein, 2009; Haynes, 2014). Si is taken up by the roots in the form of silicic acid [$Si(OH)_4$] (below pH 9), which is an uncharged molecule (Ma and Yamaji, 2006). After the absorption of Si from the soil into the root, it is translocated to the shoot, where it can deposit as a polymer of hydrated amorphous silica and can stimulate various physiological and biochemical responses (such as improved water relations and uptake of nutrients, ion homeostasis, osmolyte accumulation, enhancement in gas exchange attributes and the regulation of antioxidant enzyme activities) leading to plant growth and development (Epstein, 1999; Liang et al. 2003; Abdel-Haliem et al. 2017; Vatansever et al. 2017). Silicon plays a key role in biotic/abiotic stress alleviation through its interaction with ROS production, leading to phytohormone signalling and thus enhanced defense responses in plants (Liang et al. 2015; Frew et al. 2018a, b; Tripathi et al. 2021). Plants differ significantly in their ability to uptake and accumulate Si, ranging from 0.1 to 10.0 % Si dry weight (DW), due to differences in uptake by the roots (Ma and Yamaji, 2006). Monocot plants tend to be high Si accumulators (up to 10 % DW) compared to dicots (>1 % DW). Silicon transporters (influx and efflux) and silica cells (specialized leaf epidermal cells with the whole cell volume almost filled with solid silica) are responsible for uptake and accumulation of Si in monocot plants (Ma et al. 2006, 2010; Zargar et al. 2019). Even though dicots do not possess silica-cells, Si-transporters have been identified from several dicot plants such as soybean (Deshmukh et al. 2013), pumpkin (Mitani-Ueno et al.

2011a, b) and cucumber (Wang et al. 2014). Application of Si has been adopted as an effective strategy for alleviating the negative effects of drought stress and improving the drought stress tolerance in many plants both monocots and dicots (Gong et al. 2005; Hattori et al. 2005; Kaya et al. 2006; Chen et al. 2011; Shi et al. 2016; Ali et al. 2018). Even though few reports are available regarding the beneficial effect of Si in drought stressed legumes (Hamayun et al. 2010; Shen et al. 2010; Kurdali et al. 2013; Zhang et al. 2017), the actual biochemical mechanisms involved have not yet been demonstrated. Despite previous attempts to underpin the role of Si in plant development and growth under stress/non-stress conditions (Ma, 2004; Li et al. 2007; Coskun et al. 2016; Deshmukh et al. 2017; Etesami, 2018), the impact of Si on ROS/RNS metabolism have only partially been explored to date.

Lentil (*Lens culinaris*, Medik.) is an important legume food crop and an excellent source of proteins and nutrients, enhancing food and nutritional security (Faris et al. 2013). The growth and production of lentil is adversely affected by drought stress in many regions with Mediterranean and temperate type environments. Our previous research advocated a Si role for promoting lentil seedling growth under drought stress through the regulation of hydrolytic enzymes, osmolytes and antioxidant metabolism (Biju et al. 2017). The present study further investigates the role of Si in ROS/RNS production and its association with antioxidant defence responses in drought stressed lentil plants. Additionally, the current research aims to compare the defensive role of Si in lentil genotypes with different drought tolerant levels under different drought stress conditions.

2. Materials and Methods

2.1. Plant materials and the experimental layout

Lentil genotypes (Table 1; from The Australian Grains Gene Bank, Victoria) were selected from a non-destructive (infrared thermal imaging) drought tolerance screening experiment (Biju et al. 2018). This experiment was conducted in a growth room (Temperature: 23 ± 2°C; Relative humidity: 45-50%; Photoperiod: 12 h; Light intensity: 300-325 µmol m^2 s^{-1} from metal halide illumination lamps (MH 400W/640 E40 CLU 1SL/6, Netherlands) of the University of Melbourne, Parkville during January-August, 2018. Lentil seeds were sown, after surface-sterilization (30 % (v/v) hydrogen peroxide solution), in plastic pots (950 mL) filled with 700 g potting mix . Sodium metasilicate (Na_2SiO_3) was used as a source of silica to alleviate the adverse effects of drought stress in lentil (Biju et al. 2017), 2 mM of Na_2SiO_3 (500 mL kg^{-1} potting mix) was added to the pots before sowing. The molarity of Na_2SiO_3 was calculated based on the volume of potting mix and the pH of the solution was reduced from 8.9 to 7.5 using 0.1 N hydrochloric acid. The control pots were supplied with sodium sulfate (Na_2SO_4; 2 mM) to balance the sodium levels and the basification due to Si. Plants were fertilized with Nitrosol (Amsgrow) during the vegetative stage to maintain normal plant growth. The experimental design was completely randomised with 5 replicates for each treatment. The treatments included (1) control (C) with 100 % field capacity/FC), (2) moderate stress (MD), 50% FC), (3) moderate stress and Si (MD+Si), (4) severe stress (SD), 20% FC, (5) severe stress and Si (SD+Si), and (6) Si only (Si). Drought stress was imposed at the onset of flowering period for 28 days at respective field capacities. Furthermore, to maintain uniformity of growing conditions and elimination of light and air flow stress biasness, pots

2.2. Above-ground biomass, Relative water content (RWC) and Total chlorophyll pigments

The above-ground biomass (g) of plants from all the treatments was recorded immediately after harvesting. The leaf relative content was measured on the last day of drought stress using the following formula (Kramer, 1988):

$$RWC = [(FW - DW)/(TW - DW)] \times 100 \qquad \text{Eqn. (1)}$$

where, FW -Fresh weight, DW-Dry weight, TW -Turgid weight.

SPAD-502 (Spectrum Technologies, Inc., Aurora, Ill) which is a hand-held chlorophyll meter was used to measure total chlorophyll pigment content of the lentil genotypes on last day of drought stress.

2.3. Osmolytes (proline, glycine betaine-GB) determination

2.3.1. Proline

Proline was measured following the methodology of Bates et al. (1973). 0.1 g leaf sample was ground using 3 % aqueous sulfosalicylic acid ($C_6(OH)_5COSH$) followed by filtration of homogenised mixture using Whatman filter paper (no. 2; GE Healthcare). The total volume of the extract was made up to 10 mL using 3% ($C_6(OH)_5COSH$) solution. Acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 30 mL glacial acetic acid (CH_3COOH) and 20 mL of 6 M phosphoric acid (H_3PO_4). The reaction mixture was prepared by mixing 2 mL of filtrate with 2 mL acid-ninhydrin and 2 mL of CH_3COOH. The resulting mixture was incubated at 100°C for 1 h and the reaction was ceased in an ice bath. Subsequently, the reaction mixture was extracted using 4 mL toluene (C_6H_5-CH_3) and the absorbance was measured at 520 nm using a spectrophotometer (M501, Campspec Ltd). Proline content in the samples (µg mL^{-1}) was determined from a standard curve (linear regression with proline concentration on the x-axis and the measured absorbance at 520 nm on the Y-axis) prepared using proline. Proline (µmol g^{-1} tissue) was calculated using the following equation:

$$\frac{\mu\text{g proline/ml} \times \text{X ml toluene}}{115.5\ \mu\text{g}/\mu\text{mol}} \times \frac{\text{g sample}}{5} = \mu\text{mol proline/g fresh weight sample} \qquad \text{Eqn. (2)}$$

where, X= toluene (mL) used for the extraction of the reaction mixture, 115.5 is the molecular weight of proline and 5 is the dilution factor.

2.3.2. Glycine betaine (GB)

The estimation of GB was carried out using the method of Grieve and Grattan (1983). Extracts were prepared by continuous shaking of 500 mg powdered leaf samples with deionized water (20 mL) at 25°C for 24 h. Subsequently, the extracts were filtered and diluted in 1:1 ratio with 2 N sulphuric acid (H_2SO_4), followed by 1-hour incubation in an ice bath. Cold potassium iodide-iodine solution (KI-I_2) reagent (0.2 mL) was prepared by dissolving 15.7 g of iodine and 20.0 g of KI in 100 mL deionized water and was added to the reaction mixture. The solution (KI-I_2) was kept at 4°C for 16 h with continuous stirring before centrifugation at 12 298 g for 15 min. The supernatant was mixed with 9.0 mL of 1, 2-dichloroethane to dissolve the periodide crystals and the reaction mixture was vortexed vigorously for 2 hrs. The absorbance was taken at 365 nm using a spectrophotometer and GB concentration was calculated using the standards curve prepared using GB (50-200 µg mL^{-1}) prepared in 1N H_2SO_4.

2.4. Reactive oxygen/nitrogen species and lipid peroxidation (LPX)

2.4.1. Superoxide anion ($O_2^{\cdot-}$)

The concentration of $O_2^{\cdot-}$ was determined following Doke's methodology (1983). One-gram leaf sample was grounded with 10 mL of 50 mM sodium acetate buffer containing 10 mM sodium chloride (NaCl) (pH 6.5) in a pre-chilled mortar. The extract was filtered and centrifuged for 10 min at 12 298 g. The assay reagent [(0.01 M potassium phosphate buffer (pH 7.8) containing 0.05% nitro blue tetrazolium sodium salt (NBT) and 10 mM sodium azide

(NaN_3)] was added to the supernatant (0.1 mL) and incubated for 30 min. The initial absorbance was recorded at 580 nm using a spectrophotometer. The reaction mixture was heated at 85°C for 15 min to record the final absorbance.

2.4.2. Hydrogen peroxide (H_2O_2)

The H_2O_2 concentration was measured as per the method of Bellincampi et al. (2000). One-gram leaf sample was grounded in cold phosphate buffer (10 mM; pH 7) in a pre-chilled mortar and the homogenized mixture was filtered using Whatman filter paper. The filtrate was centrifuged for 10 min at 12 298 g. An aliquot of 1.5 mL supernatant was mixed with an equal volume of assay reagent (ammonium ferrous sulphate-500 μM, H_2SO_4-50 mM, xylenol orange-200 μM and sorbitol-200 mM). The resulting reaction mixture was first incubated at room temperature for 45 min followed by the spectrophotometer absorbance read at 560 nm.

2.4.3. Nitric oxide (NO)

The method by Hu et al. (2003) was used to estimate NO content. One-gram leaf sample was grounded in a mortar using 3 mL of 50 mM cold acetic acid buffer (pH 3.6, which had 4% zinc diacetate). Supernatant (A) was collected from homogenate and centrifuged for 15 min (12 298 g). The remaining pellet was washed with the same extraction buffer and supernatant (B) was collected after centrifugation for 15 min (12,298 g). Two supernatants (A and B) were mixed together with 0.1 g of charcoal and the filtrate was collected using Whatman filter paper. The mixture of equal volumes of filtrate (1 mL) and the Griess reagent (1 mL) was incubated for 30 min at room temperature. Spectrophotometer was used to measure the absorbance at 540 nm.

2.4.4. Lipid peroxidation (LPX)

The content of malondialdehyde (MDA) which indicates the extent of lipid peroxidation was determined according to Heath and packer's (1968) method. Leaf sample (0.5 g) was homogenised using 10 mL of 0.1% trichloro acetic acid (TCA), filtered using Whatman filter paper and centrifuged for 5 min at 27 670 g. The supernatant (0.1 mL) was mixed with 0.5% thiobarbituric acid (TBA) containing 20% TCA (4.0 mL). The resulting solution was heated for 30 min at 95°C in a boiling water bath and the reaction was immediately terminated by placing the solution in an ice bath. The reaction mixture was again subjected to centrifugation at 12 298 g for 10 min and the absorbance of the supernatant was measured at 532 nm and 600 nm. Optical density values at 600 nm were subtracted from the MDA-TBA complex values at 532 nm. MDA concentration (µmol MDA g^{-1} fresh weight) was calculated using the Lambert-Beer law with an extinction coefficient $\varepsilon M= 155\ mM^{-1}cm^{-1}$ and the formula:

$$\text{MDA } (\mu M) = (A532 - A600)/155 \times 1000$$

Eqn. (3)

2.5. Antioxidants and silicon content

2.5.1. Ascorbic Acid (ASC)

Ascorbic acid (ASC) was measured in the leaf samples using Mukherji and Chaudhari's (1983) method. One-gram of leaf sample was homogenised in 6% Trichloroacetic acid (TCA), and the homogenate was filtered using Whatman filter paper. The filtrate was subjected to centrifugation for 15 min at 3075 g. An aliquot of the supernatant (4 mL) was mixed with 2% Dinitrophenylhydrazine (DNPH) and one drop of 10% thiourea. The resulting reaction mixture was heated for 15 min using a boiling water bath and was cooled to normal room temperature. Chilled sulfuric acid (H_2SO_4) (5 mL) was added to the mixture solution at 0°C. The absorbance was taken at 530 nm and a known ascorbic acid concentration was used to draw a standard curve to estimate ASC concentration (mg g^{-1} DW).

2.5.2. Glutathione (GSH)

Glutathione (GSH) was measured in the leaf samples using Griffith's (1980) method. One-gram leaf sample was homogenised in metaphosphoric acid (HPO_3) (2 mL) and the homogenate was centrifuged for 15 min at 12 298 g. The supernatant was collected and neutralised by adding 10% sodium citrate ($Na_3C_6H_5O_7$). The reaction mixture (1 mL) was prepared by mixing 0.3 mM NADPH (700 µL), 6 mM 5,5-dithio-bis-(2-nitrobenzoic acid (DTNB; 100 µl), distilled water (100 µL) and the extract (100 µL). The assay mixture was kept for 4 min at 25°C in a water bath. Subsequently, glutathione reductase (10 µL; Sigma) was added and the absorbance was measured at 412 nm.

2.5.3. Silicon (Si)

Leaf Si content was estimated by a modified Autoclave-induced digestion (AID) method proposed by Elliot and Snyder (1991). Oven-dried leaves were grounded finely in a centrifugal mill (ZM-200, Retsch). The powered sample (0.1 g) was gently mixed with 50% sodium hydroxide (NaOH; 3.25 mL) and 30% hydrogen peroxide (H_2O_2; 3 mL) solution. The reaction mixture was autoclaved for 1 h (126°C; 138 kPa). Final volume of digested sample was adjusted to 50 mL using distilled water. Colorimetric molybdenum blue method was used to measure the Si content (Liang et al. 2015). The digested sample (1.0 mL) was mixed with 20 percent acetic acid (9 mL) and ammonium molybdate solution (2.5 mL; 54g L^{-1}, pH 7.5) in a polypropylene volumetric flask (50 mL). Subsequently, 20 percent tartaric acid and a freshly prepared reducing solution (0.25 mL) [(8 g L^{-1} sodium sulphite (Na_2SO_3) + 1.6 g L^{-1} 1-amino- 2-naphthol-4-sulfonic acid ($C_{10}H_9NO_4S$) +100 g L^{-1} sodium bisulphite ($NaHSO_3$)] were added to the sample and was kept for 30 min at room temperature. A spectrophotometer was used to record absorbance at 650 nm and the standard curve was obtained using Si standard solutions (Si 1000, Kanto Chemical Co. Inc.) to estimate the Si content.

2.6. Antioxidant enzymes

2.6.1. Superoxide dismutase (SOD: EC.1.15.1.1)

Lentil leaves (1 g) were grounded in liquid nitrogen using mortar and pestle and the crude enzyme was extracted with 100 mM potassium phosphate buffer (pH 7.6). The homogenate was centrifuged at 12 298 g for 10 min at 4°C, and the supernatant was used as the crude enzyme extract. The mixture (3 mL) was prepared by mixing the enzyme extract (0.1 mL), phosphate buffer (100 mM; pH 7.6), Na_2CO_3 (1.5 mM), NBT (2.25 mM), methionine (200 mM), ethylene diamine tetra acetic acid (EDTA; 3 mM), riboflavin (0.06 mM) and distilled water. The sample mixture reaction tubes were kept under fluorescent lamp (15 W) for 10 min along with the control samples. The absorbance of samples was read at 560 nm against the blank (a non-irradiated complete reaction mixture) using a spectrophotometer. Protein concentration was determined according to the method of Bradford (1976) using bovine serum albumin (BSA, Sigma) as standard. The SOD activity was estimated by observing the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) as described by Dhindsa et al. (1981).

2.6.2. Catalase (CAT: EC.1.11.1.6)

The CAT activity was assayed following Chance and Maehly's (1955) method. One-gram lentil leaves were grounded in a mortar and pestle, using liquid nitrogen and the crude enzyme was extracted with 50 mM sodium phosphate buffer (pH 6.8). The extract was centrifuged (12 298 g;10 min; 4°C) and the supernatant was collected. Enzyme extract (40 µL) was mixed with 30% H_2O_2 (v/v) in the same extraction buffer and the absorbance was read at 240 nm in a spectrophotometer. An equal volume of buffer containing H_2O_2 was used as the blank. Protein concentration of the sample was calculated using BSA as standard (Bradford, 1976). The molar absorption coefficient of H_2O_2 (0.04 mM^{-1} cm^{-1}) was used to calculate the enzyme activity (mM H_2O_2 mg^{-1} protein min^{-1}).

2.6.3. Peroxidase (POX: EC 1.11.1)

Peroxidase activity in the leaf samples was estimated as per Goliber's (1989) method. The crude enzyme was extracted from lentil leaves using 50 mM sodium phosphate buffer (pH 6.8). The supernatant was collected after centrifuging the extract for 10 min at 12 298 g at 4°C. The supernatant (40 µL) was added to the reaction mixture [(0.1 M phosphate buffer (pH 6.8; 0.1 mL), guaiacol (20 mM; 1 mL) and H_2O_2 (10 mM; 50 µL)]. Subsequently, POX activity was determined by monitoring the oxidation of guaiacol in the presence of H_2O_2 using spectrophotometer at 470 nm for 10 min at 30°C. Protein concentration of the samples was calculated (Bradford, 1976) to measure the specific activity (mM guaiacol mg^{-1} protein min^{-1}) of the enzyme.

2.6.4. Ascorbate peroxidase (APX: EC.1.11.1.11)

The activity of APX was estimated from lentil leaves (Chen and Asada, 1989) as a decrease in absorbance by recording the oxidation of ASC at 290 nm using a spectrophotometer. 50 mM sodium phosphate buffer (pH 7.2) was used to extract the crude enzyme from lentil leaves and subsequently, the extract was centrifuged for 10 min at 12 298 g at 4°C. The assay mixture was prepared by mixing the crude enzyme extract (100 µL), ASC (0.5 mM; 200 µL), 30% H_2O_2 (v/v)- 200 µL and the extraction buffer (500 µL). The estimated protein content of the sample (Bradford, 1976) and the molar absorption coefficient of ASC (2.8 mM cm^{-1}) were used to measure the activity of APX (mM ascorbate mg^{-1} protein min^{-1}).

2.6.5. Dehydro-ascorbate reductase (DHAR: EC.1.8.5.1)

The method proposed by Nakano and Asada (1981) was used to estimate the activity of DHAR in the leaf samples. One-gram leaf sample was grounded in liquid nitrogen using mortar and pestle the crude enzyme was extracted with 100 mM potassium phosphate buffer (pH 7.6) using polyvinyl pyrrolidone (PVP) under ice cold conditions. The extract was centrifuged for 10 min at 12 298 g at 4°C and the collected supernatant was mixed with 50 mM phosphate buffer (pH 7.0), 2.5 mM GSH and 0.1 mM dehydroacsorbate (DHA). The absorbance of enzyme extract was measured at 265 nm with a spectrophotometer and the activity was expressed as EU mg^{-1} protein.

2.6.6. Glutathione reductase (GR: EC.1.6.4.2)

The activity of GR was determined following the methodology of Mavis and Stellwagen (1968). One-gram leaf sample was homogenised in liquid nitrogen and then extracted with 100 mM potassium phosphate buffer (pH 7.6). The crude extract is subjected to centrifugation at 12 298 g at 4°C for 10 min. The assay mixture contained enzyme solution glutathione oxidised, b-NADP, bovine serum albumin (BSA), the extraction buffer and distilled water. The absorbance of the reaction mixture was read at 340 nm and the activity of GR (mMol oxidised donor min^{-1} mg^{-1} protein) was calculated using the molar extinction coefficient of b-NADP (6.2 mM^{-1} cm^{-1}).

2.6.7. Nitrate reductase (NR: EC.1.6.1.1)

Nitrate reductase activity was estimated following Reis et al.’s (2009) method. Leaves (200 mg) were cut to discs and were transferred to assay tubes with 5 mL of 100 mM potassium phosphate buffer (pH 7.5) containing 100 mM KNO_3. Subsequently, the samples in the assay tubes (wrapped in aluminium foil) were subjected to incubation in a water bath (30°C) for 60 min. The reaction mixture was prepared by mixing the supernatant (100 µL) with 1% sulfanilamide in 2 M HCl, 0.02% naphtylene diamine solution and distilled water. The rate of nitrite (NO_2^-) produced or the enzyme activity (nmol nitrite^{-1}min^{-1}mg protein) was measured in a spectrophotometer at 540 nm using a nitrite standard calibration curve. Protein content of the sample was measured following Bradford (1976) method.

2.7. Statistical analysis

A complete randomised design with five replicates for each treatment was used in the present study. Analysis of variance (ANOVA) and turkey pairwise comparison test ($P \leq 0.05$) were to compare the genotypes and treatments. Minitab®v17 (Minitab Inc.) was used to perform the statistical analysis. Multivariate analysis method based on principal component analysis (PCA), cluster analysis and covariance matrix algorithms, was performed using a customised code written in Matlab ver2017b (Mathworks Inc.) to identify the changes in drought tolerance levels of lentil genotypes with Si supplementation under severe drought stress and to determine the correlation between the variables or the studied traits.

3. Results

3.1. Aboveground biomass, relative water content and total chlorophyll pigments

Drought stressed lentil genotypes of different tolerance levels showed reduced aboveground biomass in moderate and severe drought stress treatments as compared to the control. However, application of Si alleviated the drought stress and increased the biomass by 7-23% in MD+Si and 28-50% in SD+Si treatments for all the genotypes (Fig.1A). Treatment with Si alone also significantly increased the biomass compared to control. Drought stressed plants showed significant reduction in the RWC and chlorophyll content compared to controls. However, Si application (MD+Si and SD+Si treatments) increased the RWC content and chlorophyll content of all the genotypes as compared to the control plants (Figs.1B-C).

3.2. Accumulation of osmolytes (proline and glycine betaine)

All genotypes over accumulated proline and GB under drought stress relative to the control treatment (Fig. 2). Si application regulated these osmolytes leading to considerable reduction in their values under moderate and severe drought stress conditions.

3.3. Regulation of reactive oxygen species (H_2O_2, $O_2^{\cdot -}$), reactive nitrogen species (NO) and lipid peroxidation (LPX)

Drought stress treatments led to a significant increase in the concentration of H_2O_2, $O_2^{\cdot -}$, NO and lipid peroxides in all the genotypes as compared to their respective controls. However, Si supplementation had a positive effect and significantly declined their concentration in drought stressed plants (moderate and severe drought stress treatments)

(Table 2). Furthermore, Si application to non-stressed plants also showed significant reductions in ROS/RNS contents in comparison to their respective controls.

3.4. Levels of ASC, total glutathione (GSH) and Si content.

The content of ASC, glutathione and Si in lentil genotypes increased in both moderate and drought stress treatments as compared to their respective controls. However, Si supplementation of the drought stressed genotypes resulted in further enhancement in ASC, and Si levels in all the genotypes (Fig. 3).

3.5. Antioxidant enzymes (SOD, CAT, POX, APX, DHAR, GR and NR)

Si supplied drought stressed genotypes had significantly higher antioxidant enzyme activities (SOD, CAT, POX) compared to their respective drought stressed genotypes (Fig. 4). Under Si treatment, the activity of the enzymes was also enhanced in all the genotypes compared to their respective controls.

Similar trends were observed for the activity of other antioxidant enzymes such as APX, DHAR, GR and NR in lentil genotypes. Si-treated drought stressed genotypes (MD+Si and SD+Si) and Si alone treated genotypes (Si) had significantly higher values as compared to their respective control plants for all the enzymes except NR (Fig. 5).

3.6. Multivariate data analysis (Principle component analysis, cluster diagram and covariance matrix)

The results from the principle component analysis (PCA) of severe drought stress treatments (SD) are depicted in Fig. 6. The PCA explained a total of 87.45% (PC1 = 52.64%; PC2 = 34.81%) of variance for the data (Fig. 6A). Four groups were noticed in the PCA biplot. Group 1 comprising control treatments distinctly separated into tolerant, moderately tolerant and sensitive genotypes (Fig. 6A). Genotypes in the other three treatment groups also followed the same trend of separation. The drought stress treatment without Si supplementation revealed lesser levels of the drought tolerance, deploying all the genotypes away from the origin towards the negative direction of the vectors in the PCA biplot (group 2). Group 3 corresponded to the genotypes in the Si alone treatment (Si) with tolerance levels almost similar to the control group and located near to the origin of the biplot between the D (drought stressed; group 2) and the DSi (drought stress+Si; group 4). Group 4 from the DSi treatment exhibited increased drought tolerance trait values for all the genotypes positioning the group to the extreme right towards the positive direction of the vectors in the biplot. The cluster analysis clustered different treatments into different groups based on the biochemical parameters studied (Fig. 6B). The control was separated from the drought stress treatments at a linkage distance of 55, whereas the drought stress treatments were separated from the DSi treatments at 62. The drought tolerant genotypes always assembled together under different treatments except in the Si alone treatment. Similar clustering with some exceptions was displayed by moderately drought tolerant and drought sensitive genotypes. All the antioxidant enzymes and compounds were significantly correlated among themselves (statistical significance at $P \leq 0.05$, Fig. 7). Significant positive correlations were observed between NR, SOD, CAT, GR, Glutathione, ASC, POX, APX whereas, the osmolytes (proline and GB), LPX, ROS and NO showed significant negative correlations with antioxidant enzymes (Fig. 7).

4. Discussion

4.1. Si maintains aboveground biomass, relative water content, total chlorophyll pigments and osmolytes in drought stressed lentil plants

Biomass reduction is the most common and significant effect of drought stress on plants. Current results showed that the aboveground biomass was significantly higher in all the studied lentil genotypes with added Si, both under stress and non-stress conditions (Fig. 1A), agreeing with previous findings that Si plays a role in alleviating drought stress in lentil (Biju et al. 2017). The Si-mediated positive effect on biomass could be attributed to its ability to modify cell wall metabolism by improving tissue extensibility, enhancing cell enlargement, inhibiting cell membrane deterioration (Ma and Yamaji, 2006) and improving the rate of growth and development under drought stress (Ma et al. 2004; Merwad, 2018).

The maintenance of a favourable leaf water status is an efficient adaptive mechanism for plant growth under drought stress conditions (Morgan et al. 1984). In this study, RWC declined under moderate and severe drought stress (Fig. 1B), which might be due to an imbalance between transpirational loss and water uptake by the roots. The resulting loss of turgor may have limited the cell expansion and growth in the lentil genotypes. Further, osmotic stress might have induced physiological water deficit conditions hampering water uptake as it stimulates the accumulation of osmolytes, as seen in this study. Supplementation of Si helped the lentil plants take up and retain more water and thus improve RWC under drought stress. This water restoration in drought-stressed plants could be related to the formation of a double layered silica cuticle under the leaf epidermal cells, which modifies the cell wall properties (Luyckx et al. 2017). Si supplementation could also lead to improvement of the internal ionic balances, which could regulate the water balance in plants under drought stress (Alzaharani et al. 2018). The present results support the claim by Soukup et al. (2017) that Si supplementation of Sorghum seedlings could direct the apoplastic movement of silicic acid in cell wall components (via confirmed locations of silica deposition in cell walls) and thus maintain the balance of water movement in the plant tissue. The findings of the current study are also supported by the work of Hattori et al. (2007) and Gong and Chen (2012) on the understanding of improved water status and reduced photosynthetic damage by addition of Si under drought stress in sorghum and wheat, respectively. Supplementing the drought sensitive genotypes with Si under drought stress significantly increased RWC compared to the drought tolerant ones, clearly demonstrating the beneficial effect of Si for improving the performance of drought sensitive genotypes under drought stress (Fig. 2B). The positive effect of Si on the plant water status under drought stress has also been extensively examined in various other plant species (Gunes et al. 2008; Shi et al. 2016).

Reduction in the chlorophyll pigment content has been observed in plants under various environmental stresses depending upon the degree and duration of the stress (Chaves et al. 2009). However, exogenous application of Si improved the water relations and enhanced the content of chlorophyll pigments in all the lentil genotypes under drought stress thereby facilitating a constant supply of assimilates to the growing tissues (Fig.1C). The role of Si in enhancing the content of chlorophyll pigment under drought stress has also been reported in previous studies (Ma et al. 2004; Gong et al. 2005; Shen et al. 2010). Interestingly, one of the drought tolerant genotypes, G2 (Indianhead) showed an increase in chlorophyll content under severe drought stress as compared to moderate drought stress when supplied with Si. It can be assumed that Si, in conjunction with severe drought stress, might have triggered the Type C stay-green behaviour (Thomas and Howarth, 2000) of this genotype (Data unpublished).

The accumulation of compatible osmolytes, proline and GB, under drought stress has been reported to be a noble indicator of abiotic stress tolerance in plants (Ashraf and Foolad, 2007). Proline and GB (also ROS scavengers) aids in the osmotic adjustment and restoration of chlorophyll pigment molecules under stress conditions (Kishor et al. 2005; Chen and

Murata, 2008). The decrease in proline level in drought stressed (MD and SD) lentil genotypes with Si treatment suggested three possibilities; that either (1) Si balanced the cytosol and vacuole osmotic strengths and osmotic strength of external environment (2) Si protected the cells from the oxidative burst by scavenging ROSs, (3) Si affected the activity of Δ1-pyroroline-5-carboxylate synthetase (P5CS) and proline dehydrogenase (PDH), two key enzymes which take part in proline synthesis and degradation, respectively or (4) Si reacted with the osmoprotectant, proline to form silaproline, like the mechanism occurring in humans (Vivet et al. 2000). Similar results were also found in cucumber where Si is reported to decrease the proline content under drought stress (Ouzounidou et al. 2016). In parallel, we observed that Si reduced the GB accumulation in lentils exposed to MD and SD, justifying the role of GB in osmotic adjustment and protection of the thylakoid membrane, thereby maintaining chlorophyll pigments and alleviating the drought stress effects. Thus, these findings suggest that Si exerts significant effects on the osmotic adjustments through increased water potential and water content, crucial for growth and redox balance at low water potential.

4.2. Si maintains ASC, total glutathione (GSH) and Si content in drought stressed lentil plants

ASC and GSH function as major redox buffers in the ascorbate-glutathione pathway to mediate the removal of H_2O_2 by transferring electrons from Nicotinamide adenine dinucleotide phosphate (NADPH) to H_2O_2 (Sofo et al. 2010). A close analysis of the ascorbate-glutathione aided defense mechanism under Si supplementation revealed that Si further increased the ASC and GSH level in drought stressed lentil genotypes, which might have contributed to efficient ROS scavenging and lowering LPX through increased activity of GR and POX (Figs. 3A and 3B). Along with the regulation of osmolytes, Si supplement to drought stressed plants also resulted in slower degradation of ascorbate and glutathione, thus showing a possibility of the involvement of various factors in Si induced drought tolerance.

Lentil has low levels of Si with a maximum content in the plant of less than 5% per dry weight (Biju et al. 2017). In this study, Si content increased significantly in the drought stressed lentil genotypes supplemented with Si than the genotypes subjected to moderate or severe drought stress treatments (Fig. 3C). Si interacts and binds with cell wall components such as polysaccharides, lignin and pectin, thereby affecting the mechanical properties of the cell wall like its architecture, rigidity and elasticity (Currie and Perry, 2007). This in turn leads to electrolyte leakage and altered water permeability of cell walls as observed in rice (Agarie et al. 1998) and maize seedlings (Kaya et al. 2006). The increased deposits of Si in cell walls under moderate and severe drought stress treatments could strengthen the plant membranes and regulate their permeability by maintaining optimal membrane fluidity and regulating the stress-dependent peroxidation of membrane lipids, enhancing drought stress tolerance, as reported by Liang (1999) in salt-stressed barley in response to Si treatment.

4.3. Si regulates the nitro-oxidative homeostasis in lentil plants under drought stress by detoxifying reactive oxygen species ($O_2^{\cdot -}$, H_2O_2), and regulating reactive nitrogen species (NO) and lipid peroxidation (LPX)

To further evaluate the role of Si in alleviating drought stress induced oxidative injuries in lentil plants, the present study examined components of the nitro-oxidative homeostasis. Drought stress leads to a disturbance in plant metabolism and causes oxidative injuries by enhancing the production of ROS (Foyer et al. 1994). The reduction of oxidative damage via decreased production of ROS and/or increased activity of antioxidant metabolism appears to play an important role in Si-induced abiotic stress alleviation in plants (Shen et al. 2010; Kim et al. 2017). The ROS accumulation in plant cells under stress will speed up the lipid peroxidation by virtue of oxidation of unsaturated fatty acids, causing membrane damage and electrolyte leakage (Gill and Tuteja, 2010). The increased activity of antioxidant enzymes and the enhanced production of non-enzymatic antioxidant components like ASC and glutathione are essential for the efficient scavenging of ROS and alleviating the oxidative damage in plants (Mittler, 2002). In the current study, exogenous Si significantly decreased the production of ROS along with enhancement in production of ASC and glutathione in all the lentil genotypes under drought stress (Figs. 3A and B), which resulted in lower lipid peroxidation (Table 2), indicating that Si contributes to the improvement of the cell membrane structure and integrity under drought stress.

Like ROS, reactive nitrogen species (RNS) are primarily produced in various cellular organelles (mitochondria, peroxisomes, plastids and bacteroides), especially under stress conditions (Corpas et al. 2008; Kausar and Shahbaz, 2013). NO helps accumulate GB in stressed plants, which in turn scavenges stress-induced ROS (Ullah et al. 2016). A significant positive correlation was observed between NO and GB in the present study (Fig. 7). The mitigating effects of Si supplementation in drought-stressed lentil plants were also evidenced by the suppression of NO content in lentil genotypes under moderate and severe drought stress in response to Si (Table 2). Thus, the present result reflects the possibility of Si mediated NO suppression in drought stressed lentil plants in alleviating the negative effects of drought stress.

4.4. Si application enhances the activities of antioxidant enzymes (SOD, CAT, POX, APX, DHAR, GR) and NR

The chief enzymatic network that detoxifies ROS is composed of SOD, CAT, POX, APX, DHAR, and GR (Mittler, 2002). SOD is believed to serve as a frontline antioxidant defense against various environmental stress regimes. SOD detoxifies $O_2^{\cdot -}$ resulting in the formation of H_2O_2 , which in turn damages chloroplasts, nucleic acids and proteins). Peroxidase plays a role in decreasing the accumulation of H_2O_2, eliminating MDA (malondialdehyde), resisting cell peroxidation of membrane lipids and maintaining the cell membrane integrity (Eshdat, 1997). In the present study, Si application to plants under moderate and severe drought stress further increased the activity of SOD, CAT and POX, which in turn reduced the overproduction of ROS, reducing the oxidative stress (Fig. 4). The effect of Si on the antioxidant enzyme activity under stress agreed with the findings of Gong et al. (2005) and Shi et al. (2016), who noted that an increase in SOD and CAT activity in drought-stressed plants with Si supplementation could significantly increase its defensive capability against oxidative damage.

The current research presented higher accumulation of H_2O_2 even after enhanced activities of the ASC-GSH cycle enzymes, proposing that the accumulation of H_2O_2 exceeded the ROS-scavenging potential of the drought stressed lentil plants (Table 2). However, the application of Si modulated the ASC-GSH glutathione cycle differentially by maintaining the activity of APX, DHAR and GR above the non-stressed control level (Fig. 5). APX plays the important role of dismutating H_2O_2, to H_2O and $O_2^{\cdot -}$. DHAR, and GR can provide substrate for APX by catalyzing the reaction and this process regenerates ASC (Asada, 1994). In the present study, the activity of APX, DHAR, and GR was increased in drought stressed plants with added Si. Furthermore, supplementation of Si improved the GR activity, maintained the redox status, possibly by generating more glutathione (Table 2), similar what has been reported in wheat plants (Gong et al. 2005).

The enzymatic activity of NR, the key biosynthetic enzyme involved in the generation of NO in plants (Yamasaki and Sakihama, 2000; Fresneau et al. 2007), displayed a similar overall pattern to that of NO (Fig. 5D), thus providing biochemical support for the declining NO content. Furthermore, the regulated levels of osmolytes in plants under moderate and severe drought stress helped maintain the catalytic activity of NR. Drought stress caused comparatively higher increment in the levels of LPX, ROS and NO in drought sensitive plants compared to drought tolerant and moderately drought tolerant genotypes under stress. Moreover, the reduction in these parameters in response to Si was more enhanced in the drought sensitive genotypes as compared to the drought tolerant and moderately drought tolerant genotypes. The actual physiological and biochemical mechanisms behind this differential response of tolerant and sensitive lentil genotypes to Si are ambiguous. However, it could be related to the genetic framework of the plants as well as the site of Si action within the plant cell.

4.5. PCA, cluster analysis and correlation matrix

The PCA results obtained from this study agree with the principle put forward by Sneath and Sokal (1973), who demonstrated that data should establish at least 70% of the total data variance (Fig. 6A). The biplot categorised the genotypes into four separate groups depending on their drought tolerance, as previously mentioned. The genotypes in group 1 displayed a normal response of drought-tolerance as expected, although the group 2 genotypes exhibited less drought tolerance with induced stress treatment. Interestingly, the group 3 and 4 genotypes showed positive effects of Si in enhancing the drought tolerance, and additionally, the results also demonstrated that Si supplementation can be used to accelerate plant growth under drought stress and non-drought stress conditions by improving the response of moderately drought tolerant and sensitive genotypes. The distinct group formation observed with the cluster analysis also supports the results from the PCA biplot (Fig. 6B). The positive and significant correlations ($P \leq 0.05$) noticed among the studied biochemical traits, such as NR, SOD, CAT, GR, Glutathione, ASC, POX, APX (Fig. 7), shows that these traits can be used to evaluate the drought-tolerance in plants. Moreover, the significant negative correlations noted between osmolytes (proline and GB), LPX, ROS and NO with the antioxidant enzymes strongly reinforces the prospective role of Si in the effective ROS/RNS scavenging in drought stressed lentil genotypes.

The current findings provide compelling evidence for the protective mode of action of Si against drought stress through the regulation of nitro-oxidative homeostasis and antioxidant metabolism in the lentil genotypes (Fig.). Si regulates ROS overproduction in drought stressed lentil plants by decreasing ROS/RNS production through the improvement of the antioxidant enzyme activities and the lowering of the lipid peroxidation. Si may help to maintain the integrity of cell membranes and lower their permeability by decreasing malondialdehyde (MDA) content, the end-product of lipid peroxidation. Regulation of osmolytes and antioxidants with Si supplementation could be an efficient strategy to increase plant tolerance to oxidative stress under drought stress.

5. Conclusions

In conclusion, the supplementation of Si enhanced the tolerance of all the contrasting lentil genotypes under drought stress with a proportionately greater beneficial effect (1) in drought sensitive plants than the tolerant plants and (2) under severe drought than under moderate drought stress conditions. The present results also advocated that Si supplementation of lentil genotypes mitigated drought stress, which in turn harmonized with the biochemical activities of the enzymatic and non-enzymatic antioxidants and the production of ROS/RNS in cells. This ultimately led to osmotic adjustment, increased water uptake and increased biomass. Silicon enhanced the normal growth and development even under non-drought stress conditions, thus highlighting its essentiality as a plant nutrient. Research conducted under field conditions validated the findings from this study and the results are under publication. Future studies are needed for investigating the use of transcriptomics and metabolomics to provide important insights into underlying mechanisms of Si mediated stress alleviation, for underpinning the role of Si in higher plant growth and development, and therefore its ability to enhance plant tolerance to drought stress.

Author contribution statement

Design of the experiment- SB, DG and SF; Execution of the experiment-SB; Collection of the data-SB; Analysis and interpretation of the data-SB, DG and SF; Preparation of the manuscript-SB; Review and editing of the manuscript-SB, DG and SF.

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Data availability statement

Data sharing is not applicable to this article as all new created data is already contained within this article.

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Legends of figures

Figure 1

Fig. 1 (A) Above ground biomass, (B) relative water content and (C) total chlorophyll pigments in lentil genotypes (G-ILL 6002, G2-Indianhead, G3-PBA Jumbo 2, G4-Nipper, G5-Flash, G6- PI 468898 and G7-ILL 7537) under different drought stress treatments (C-control, MD-moderate drought stress, MD+Si-moderate drought stress+Si , SD- severe drought stress, SD+Si-severe drought stress+Si and Si-Si alone). Mean values provided with error bars represent the standard error and different letters represent statistical significances within the genotypes (Tukey test; $P \leq 0.05$).

Figure 2

Fig. 2 (A) Proline and (B) glycine betaine in lentil genotypes (G-ILL 6002, G2- Indianhead, G3PBA Jumbo 2, G4-Nipper, G5-Flash, G6PI 468898 and G7-ILL 7537) under different drought stress treatments (C- control, MD- moderate drought stress, MD+Si-moderate drought stress+Si , SD-severe drought stress, SD+Si-severe drought stress+Si and Si-Si alone). Mean values are provided with error bars represent the standard error and different letters represent statistical significances within the genotypes (Tukey test; $P \leq 0.05$).

Figure 3

Fig. 3 (A). Ascorbate (B) glutathione and (C) silicon content in lentil genotypes (G1-ILL 6002, G2-Indianhead, G3-PBA Jumbo 2, G4-Nipper, G5-Flash, G6-PI 468898 and G7-ILL 7537) under different drought stress treatments (C-control, MD-moderate drought stress, MD+Si-moderate drought stress + Si , SD- severe drought stress, SD+Si-severe drought stress+Si and

Si-Si alone). Mean values provided with error bars represent the standard error and different letters represent statistical significances within the genotypes (Tukey test; $P \leq 0.05$).

Figure 4

Fig. 4 (A) Superoxide dismutase (SOD) (B) Catalase-CAT and (C) Guaiacol peroxidase (POX) in lentil genotypes (G1-ILL 6002, G2-Indianhead, G3- PBA Jumbo 2, G4-Nipper, G5- Flash, G6- PI 468898 and G7-ILL 7537) under different drought stress treatments (C- control, MD-moderate drought stress, MD+Si-moderate drought stress+Si , SD-severe drought stress, SD+Si-severe drought stress+Si and Si-Si alone). Mean values provided with error bars represent the standard error and different letters represent statistical significances within the genotypes (Tukey test; $P \leq 0.05$).

Figure 5

Fig. 5 (A) Ascorbate peroxidase-APX (B) dehydroasorbate reductase-DHAR (C) glutathione reductase-GR and (d) nitrate reductase-NR in lentil genotypes (G1-ILL 6002, G2-Indianhead, G3-PBA Jumbo 2, G4-Nipper, G5-Flash, G6-PI 468898 and G7-ILL 7537) under different drought stress treatments (C- control, MD-moderate drought stress, MD+Si-moderate drought stress+Si , SD-severe drought stress, SD+Si-severe drought stress+Si and Si-Si alone). Mean values provided with error bars represent the standard error and different letters represent statistical significances within the genotypes (Tukey test; $P \leq 0.05$).

Figure 6

Fig. 6. Multivariate data analysis showing: A) principal components analysis in which x-axis represents principal component one (PC1) and y-axis represents principal component two (PC2), for drought-tolerance related traits as vectors and cluster analysis according to the effect of Si on the seven lentil genotypes (G1-ILL 6002, G2-Indianhead, G3-PBA Jumbo 2, G4-Nipper, G5-Flash, G6-PI 468898 and G7- ILL 7537) under severe drought stress treatment (C-control, D-drought stress, DSi-drought stress+Si and Si-Si alone).

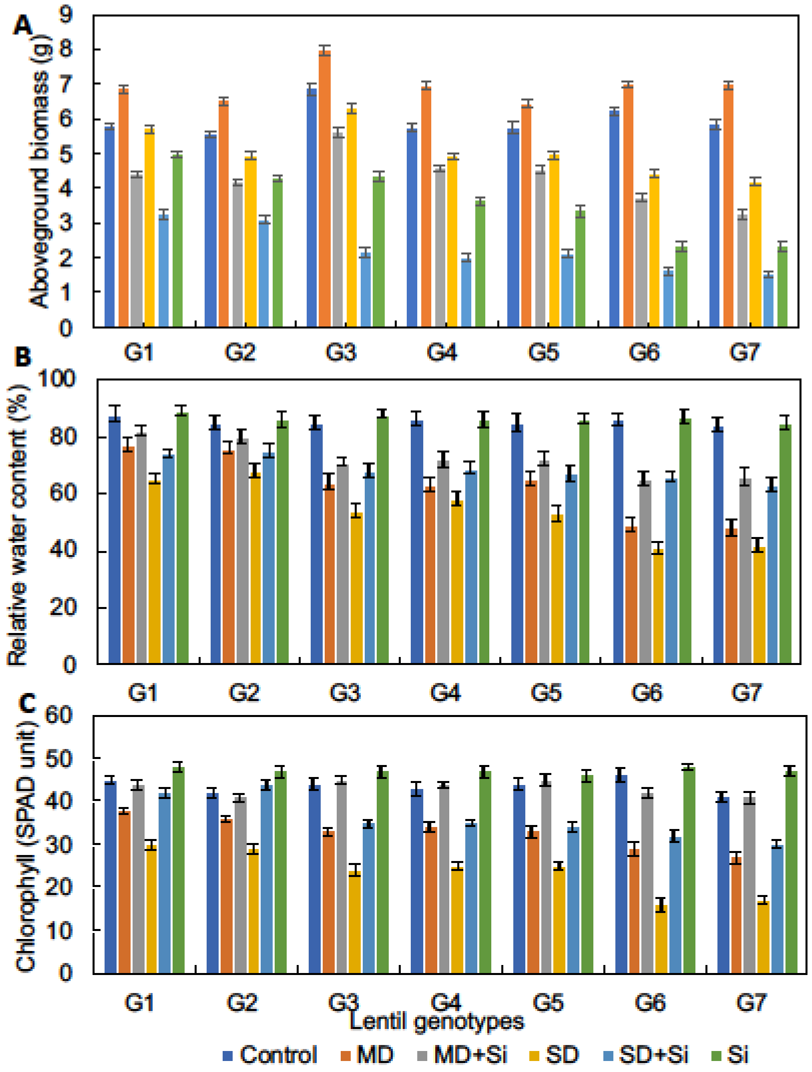
Figure 7

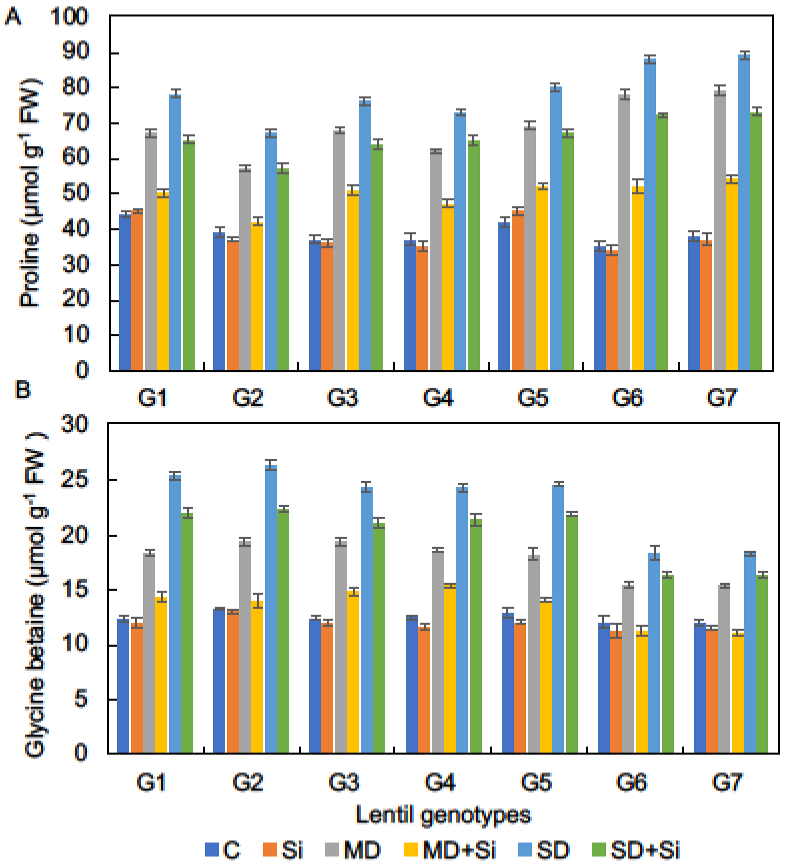
Fig. 7 Correlation matrix with significant positive and negative correlations ($P < 0.05$) according to the effect of Si on the seven lentil genotypes (G1-ILL 6002, G2-Indianhead, G3-

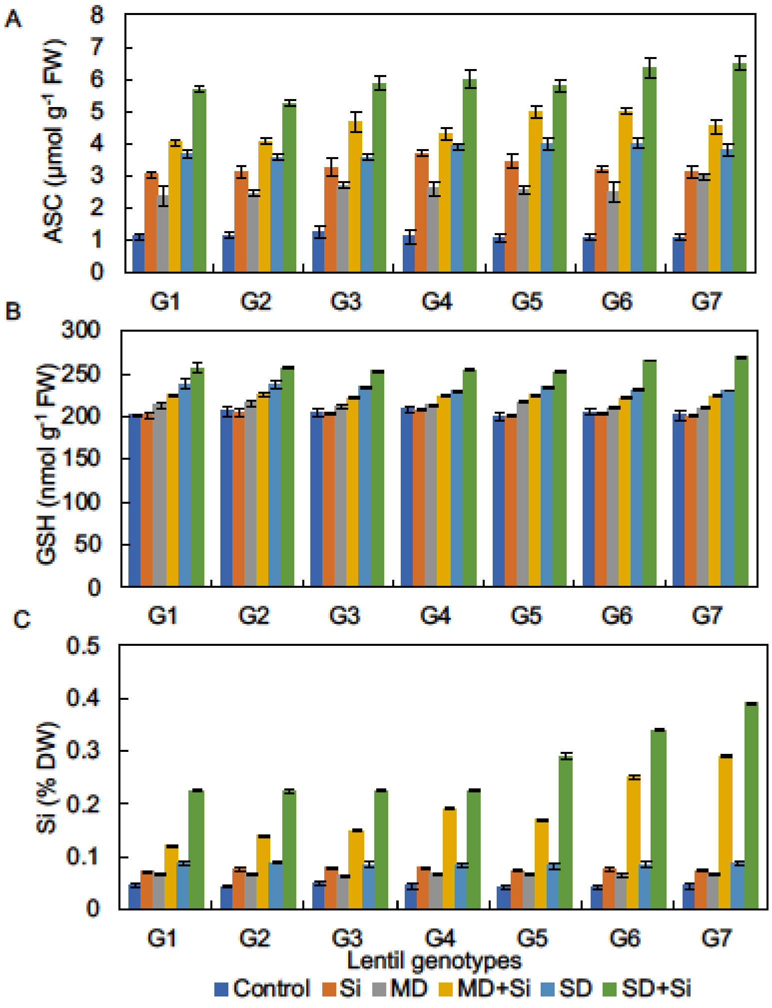
PBA Jumbo 2, G4-Nipper, G5-Flash, G6-PI 468898 and G7- ILL 7537) under severe drought stress treatment (C- control, D-drought stress, DSi-drought stress+Si and Si-Si alone).

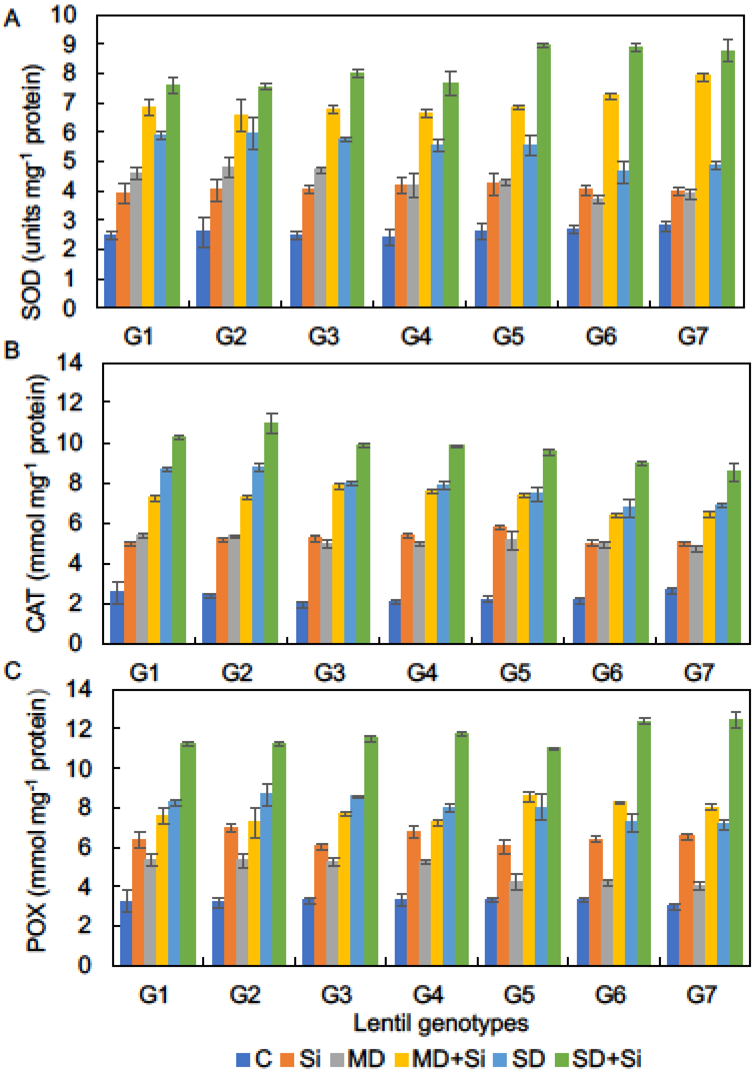
Figure 8

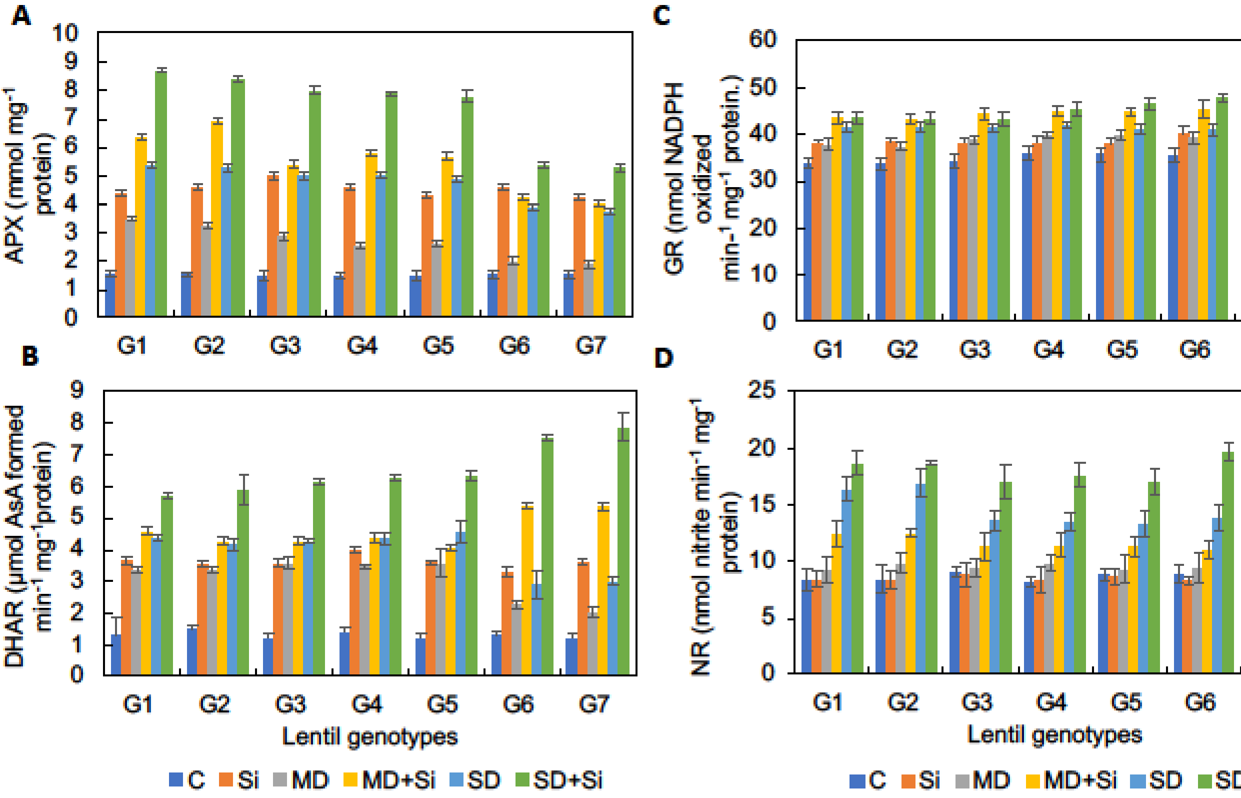
Fig. 8. Schematic diagram representing the interaction of Si with reactive oxygen/nitrogen species and antioxidant components in lentil genotypes under drought stress

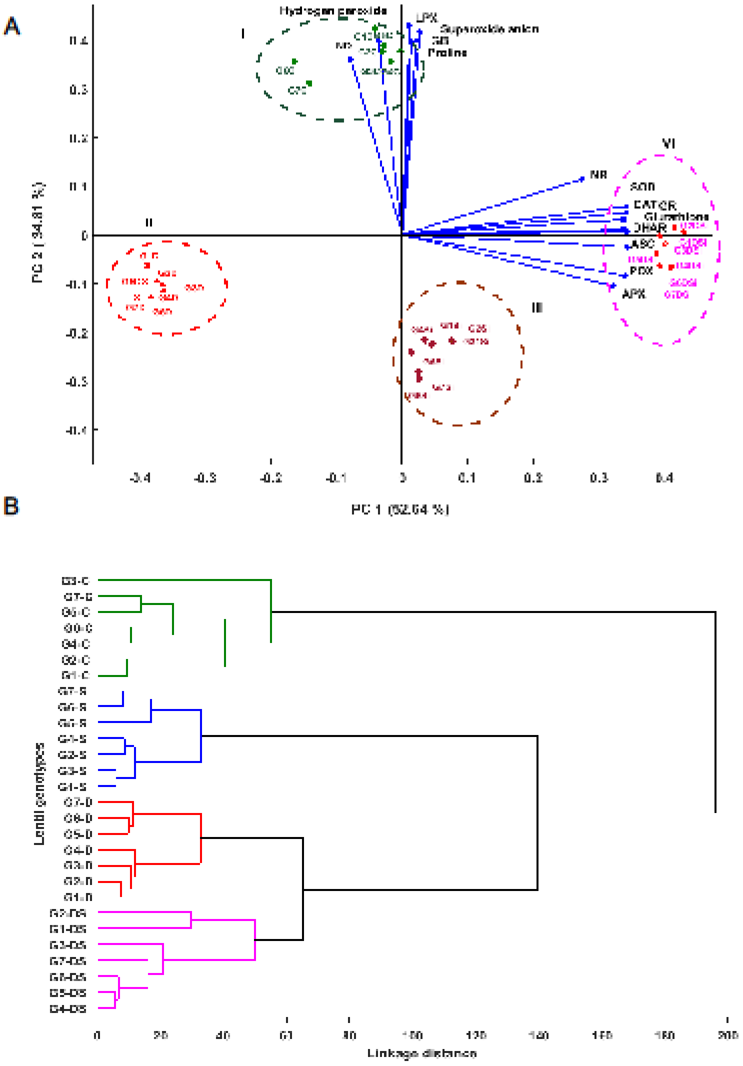

A
Aboveground biomass (g)
0
1
2
3
4
5
6
7
8
9
G1
G2
G3
G4
G5
G6
G7
B
Relative water content (%)
0
20
40
60
80
100
G1
G2
G3
G4
G5
G6
G7
C
Chlorophyll (SPAD unit)
0
10
20
30
40
50
60
G1
G2
G3
G4
G5
G6
G7
Lentil genotypes
Control
MD
MD+Si
SD
SD+Si
Si

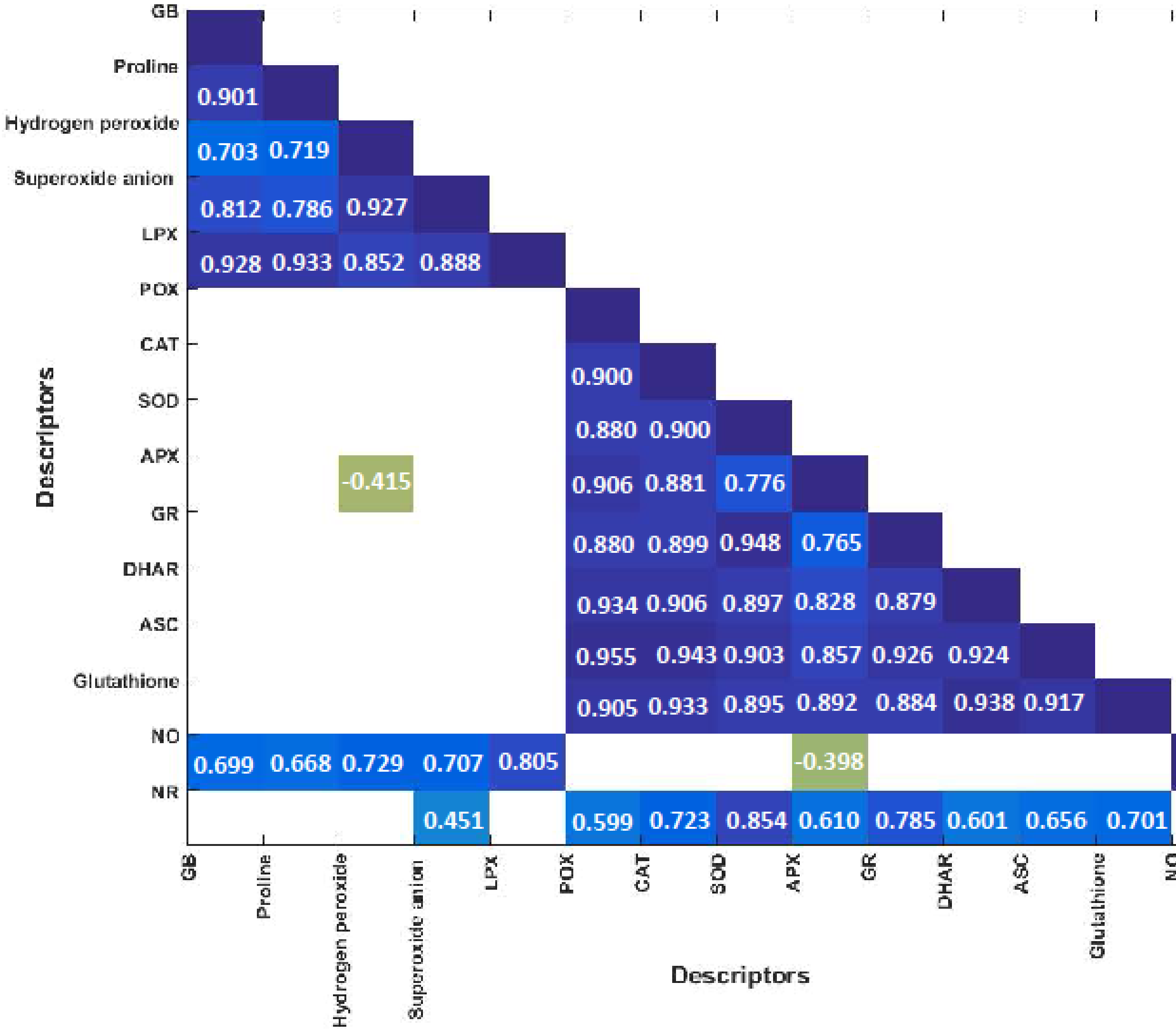

A
100
90
80
70
60
50
40
30
20
10
0
Proline (µmol g-1 FW)
G1
G2
G3
G4
G5
G6
G7
B
30
25
20
15
10
5
0
Glycine betaine (µmol g-1 FW)
G1
G2
G3
G4
G5
G6
G7
Lentil genotypes
C
Si
MD
MD+Si
SD
SD+Si


A
ASC (µmol g-1 FW)
0
1
2
3
4
5
6
7
8
G1
G2
G3
G4
G5
G6
G7
B
GSH (nmol g-1 FW)
0
50
100
150
200
250
300
G1
G2
G3
G4
G5
G6
G7
C
Si (% DW)
0
0.1
0.2
0.3
0.4
0.5
G1
G2
G3
G4
G5
G6
G7
Lentil genotypes
Control
Si
MD
MD+Si
SD
SD+Si

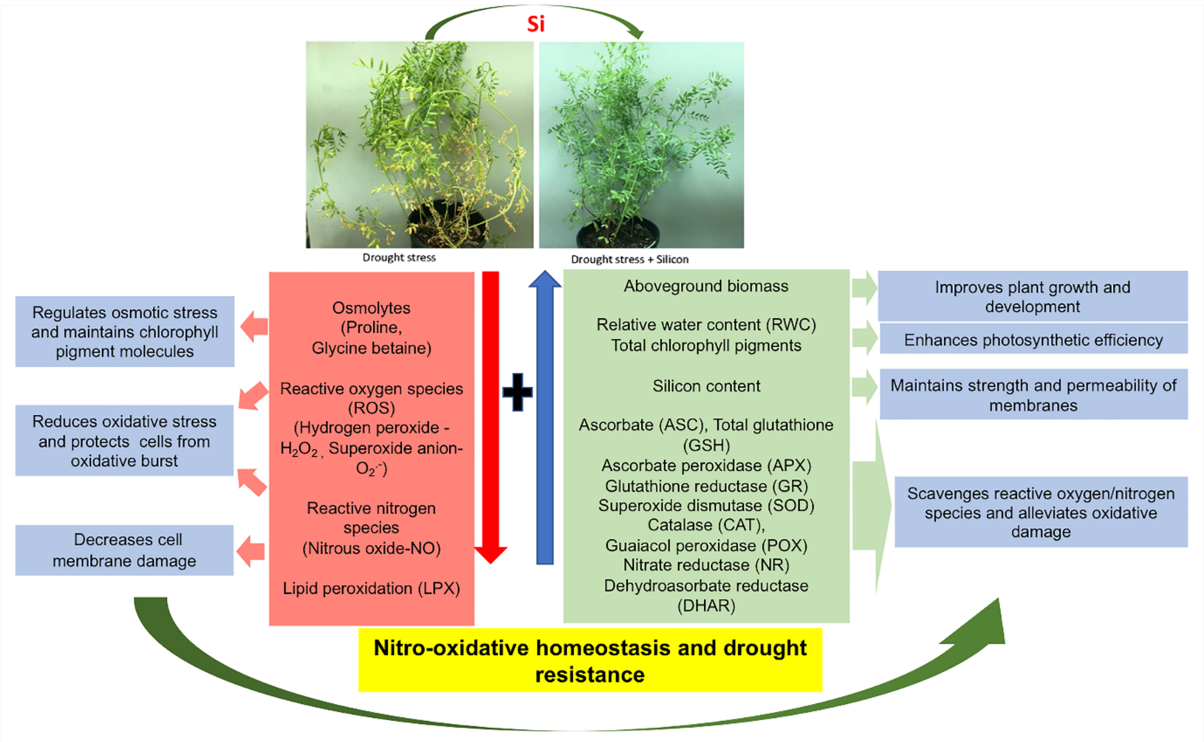

A
SOD (units mg^{-1} protein)
0
1
2
3
4
5
6
7
8
9
10
G1
G2
G3
G4
G5
G6
G7
B
CAT (mmol mg^{-1} protein)
0
2
4
6
8
10
12
14
G1
G2
G3
G4
G5
G6
G7
C
POX (mmol mg^{-1} protein)
0
2
4
6
8
10
12
14
G1
G2
G3
G4
G5
G6
G7
Lentil genotypes
C
Si
MD
MD+Si
SD
SD+Si


A
APX (mmol mg-1 protein)
10
9
8
7
6
5
4
3
2
1
0
G1
G2
G3
G4
G5
G6
G7
B
DHAR (µmol AsA formed min-1 mg-1protein)
9
8
7
6
5
4
3
2
1
0
G1
G2
G3
G4
G5
G6
G7
Lentil genotypes
C
Si
MD
MD+Si
SD
SD+Si
C
GR (nmol NADPH oxidized min-1 mg-1 protein.)
60
50
40
30
20
10
0
G1
G2
G3
G4
G5
G6
D
NR (nmol nitrite min-1 mg-1 protein)
25
20
15
10
5
0
G1
G2
G3
G4
G5
G6
Lentil genotypes
C
Si
MD
MD+Si
SD
SD


A
Hydrogen peroxide
LPX
Superoxide anion
GR
Proline
NR
SOD
CAT GR
Glutathione
DHAR
ASC
POX
APX
I
II
III
VI
G6C
G7C
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
PC 2 (34.81 %)
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4
PC 1 (52.64 %)
B
G3-C
G7-C
G5-C
G6-C
G4-C
G2-C
G1-C
G7-S
G6-S
G5-S
G4-S
G2-S
G3-S
G1-S
G7-D
G6-D
G5-D
G4-D
G3-D
G2-D
G1-D
G2-DS
G1-DS
G3-DS
G7-DS
G6-DS
G5-DS
G4-DS
Lentil genotypes
0
20
40
60
80
100
120
140
160
180
200
Linkage distance



	GB	Proline	Hydrogen peroxide	Superoxide anion	LPX	POX	CAT	SOD	APX	GR	DHAR	ASC	Glutathione
GB													
Proline	0.901												
Hydrogen peroxide	0.703	0.719											
Superoxide anion	0.812	0.786	0.927										
LPX	0.928	0.933	0.852	0.888									
POX													
CAT						0.900							
SOD						0.880	0.900						
APX			-0.415			0.906	0.881	0.776					
GR						0.880	0.899	0.948	0.765				
DHAR						0.934	0.906	0.897	0.828	0.879			
ASC						0.955	0.943	0.903	0.857	0.926	0.924		
Glutathione						0.905	0.933	0.895	0.892	0.884	0.938	0.917	
NO	0.699	0.668	0.729	0.707	0.805				-0.398				
NR				0.451		0.599	0.723	0.854	0.610	0.785	0.601	0.656	0.701


Si
Drought stress
Drought stress + Silicon
Regulates osmotic stress and maintains chlorophyll pigment molecules
Reduces oxidative stress and protects cells from oxidative burst
Decreases cell membrane damage
Osmolytes (Proline, Glycine betaine)
Reactive oxygen species (ROS) (Hydrogen peroxide - H_2O_2, Superoxide anion- $O_2^{\cdot-}$)
Reactive nitrogen species (Nitrous oxide-NO)
Lipid peroxidation (LPX)
Aboveground biomass
Relative water content (RWC)
Total chlorophyll pigments
Silicon content
Ascorbate (ASC), Total glutathione (GSH)
Ascorbate peroxidase (APX)
Glutathione reductase (GR)
Superoxide dismutase (SOD)
Catalase (CAT),
Guaiacol peroxidase (POX)
Nitrate reductase (NR)
Dehydroasorbate reductase (DHAR)
Improves plant growth and development
Enhances photosynthetic efficiency
Maintains strength and permeability of membranes
Scavenges reactive oxygen/nitrogen species and alleviates oxidative damage
Nitro-oxidative homeostasis and drought resistance