Ameliorating impacts of exogenously-applied alpha lipoic acid on growth and yield of differentially drought tolerant mungbean genotypes: photosynthetic pigments, lipid peroxidation, antioxidative defence mechanism (#92451)

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Ameliorating impacts of exogenously-applied alpha lipoic acid on growth and yield of differentially drought tolerant mungbean genotypes: photosynthetic pigments, lipid peroxidation, antioxidative defence mechanism

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Context Exogenous use of potential organic compounds through different modes is a promising strategy for the induction of water stress tolerance in crop plants for better yield. **Aims** Present study was aimed to explore the possible role of alpha lipoic acid (ALA) for the induction of water stress tolerance in mungbean genotypes when applied exogenously through different modes. **Methods** Experiment was conducted in field with split split plot arrangement having three replicates for each treatment. Two levels of water stress including normal irrigation and water deficit irrigation were applied. The plants allocated to water stress were irrigated only at the reproductive stage. Three levels of ALA (0mM, 0.1mM & 0.15mM) were applied through different modes (priming, foliar or priming+foliar). **Key results** ALA treatment through different modes manifested higher growth both under water stress and non-stress conditions. Compared to other two modes, application of ALA as seed priming was found more effective in ameliorating the adverse impacts of water stress on growth and yield that associated with their better content of leaf photosynthetic pigments, plant water relations, levels of non-enzymatic antioxidants, improved activities of enzymatic antioxidants, and decreased lipid peroxidation and H_2O_2 levels. **Conclusions** Conclusively, 0.1 and 0.15mM levels of ALA as seed priming than other modes were found better to obtain better yield of mungbean plants under deficit irrigation with better drought tolerant induction by improving physio-biochemical mechanisms. **Implications** The findings are useful for the farmers working in arid and semi-arid regions to obtain the better yield of mungbean

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ABSTRACT

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Conclusively, 0.1 and 0.15mM levels of ALA as seed priming than other modes were found better to obtain better yield of mungbean plants under deficit irrigation with better drought tolerant induction by improving physio-biochemical mechanisms.

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Keywords: Antioxidation; Seed yield; Lipoic acid; Exogenous application; Deficit irrigation; Mungbean

INTRODUCTION

Vigna radiata L. commonly named as mungbean is known as golden or green gram because of its high protein contents in its seeds that are being used highly in sprouted or as dry seed form. Mungbean is the third most important legume grain after pigeon pea and chickpea (*Bangar et al., 2019*; *Mujahid et al., 2022*). It is cultivated predominantly across the Asian countries and has also expanded to some parts of South America, Australia and Africa (*Nair et al., 2019b*; *Pratap et al., 2020*). It is fast growing, self-pollinating, diploid and short duration crop. It is helpful as effective utilization of summer fellows to increase crop production and cropping intensity (*Singh et al., 2016*). *Vigna radiata* seeds contain antifungal, antibacterial and anticancer properties (*Turk et al., 2018*; *Uppalwar et al., 2021*). Mungbean has low input requirements and a wider adaptability (*Khalid et al., 2019*; *Singh et al., 2022*). As a leguminous crop it has a strong root system helpful in the atmospheric nitrogen fixation in soil (about 58-109 kg/ha) with Rhizobium (*Allito et al., 2015*; *Lindstrom & Mousavi*, *2020*). Therefore, it plays an essential role in sustaining productivity and improving of soil fertility (Favero *et al*. 2021). It is an excellent source of antioxidants like phenolics and flavonoids. It is also a rich source of micronutrients and vegetable proteins (*Guo et al., 2012*; *Nair et al., 2015a*; *Foyer et al., 2016*) and hence has use in multifarious food (Arnoldi *et al*. 2014; Ebert 2014) and green gram manure (*Boelt et al., 2015*). Water stress is one of the principal environmental stresses that hinders plant growth, development, and crop productivity; especially in arid and semi-arid environments.

The deleterious impacts of water stress include reduction in plant growth, disruption of photosynthetic pigments, reduction in water and nitrogen use efficiency and abnormalities in cell structure. It also modifies the activities of cellular metabolic (*Chen et al., 2019*). Moreover, water stress causes over accumulation of reactive oxygen species (ROS) resulting in oxidative damages resulting in many adverse impacts including, stomatal closure; altered activities of cellular enzymes results in reduced photosynthesis. Generation of ROS also causes membrane lipid peroxidation and subsequently it damages the membrane and also damages the proteins and nucleic acids (*Maksup et al., 2012*).

Plants have developed a variety of defense mechanisms to counteract the ROS induced damages, such as increased accumulation of non-enzymatic and activities of enzymatic antioxidant including glutathione, ascorbic acid, carotenoids, tocopherols, CAT, ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and SOD (*Abd-Elhamid et al., 2014*). Different phenolic compounds are also well-known antioxidants enhancing oxidative stress tolerance in plant tissues (*Chen et al., 2019*). Though the plants have well known mechanism to cope the stresses but its extent of defense is plant specific and type of stress specific. Different ways are being used to enhance plant stress tolerance against different stresses. It has been reported that the application of effective, affordable, and cheap chemicals is found effective to enhance plant tolerance to biotic and abiotic stress, including the water stress. Amongst these, alpha lipoic acid is also considered as one of the novel substances among others but few reports are available

Alpha lipoic acid [formula, $C_8H_{14}O_2S_2$, molar mass 206.33g/mol] is a small molecule of disulfide, which act as co-enzyme of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase in mitochondria. It was first isolated from the liver by Reed in 1951 and discovered in 1973 (*Reed et al., 1951*). Itis found in both eukaryotic and bacterial organisms (*Terzi et al., 2018*). ALA and its reduced form, dihydrolipoic (DHLA) have powerful antioxidant potential which could reduce the ROS levels and free radicals, chelate metal ions, weaken oxidative stress, promote the endogenous antioxidant's regeneration such as coenzyme Q10, glutathione as well as its own levels (*Huang & Huang, 2004*; *Liu et al., 2005*; *Alban et al., 2018*). Moreover, ALA is the only known antioxidant that has both lipo-soluble and water-soluble properties. Unlike liposoluble antioxidants which only act on the cell membrane, and unlike water soluble antioxidant which only act on cytoplasm, ALA plays a dual protection role that can function simultaneously on cell membrane as well as the cytoplasm (*Çelik & Ozkaya, 2002*; *Mian et al., 2021*).

Due to its two sulfhydryl moieties, it binds with metals and enable them to scavenge the free radicals, that are responsible for its antioxidant capacity (*Fogacci et al., 2020*). Exogenous ALA application has been found to mitigate the lipid peroxidation regulates the osmotic potential and leaf photosynthetic performance under abiotic stresses (*Gorcek & Erdal, 2015*; *Sezgin et al., 2019*; *Elkelish et al., 2021*; *Youssef et al., 2021*). Exogenous use of ALA increases the enzymatic antioxidant activities such as monodehydro ascorbate reductase (MDHAR), glutathione reductase (GR), GPX, CAT and SOD under osmotic stress (*Perez-Lopez 2010*; *Gorcek, & Erdal, 2015*; *Terzi et al., 2018*). Additionally, ALA has been found involved in the restoration of grain yield and quality attributes of water stressed wheat plants (Elkelish *et al*., 2021). Under diverse environmental stress ALA has found helpful for induction of stress tolerance mechanisms in several plant species by improving the photosynthesis (*Turk et al., 2018*; *Terzi et al., 2018*; *Sezgin et al., 2019*).

Only few reports regarding the role of ALA in the alleviation of negative impacts of water deficit stress are available and only are in the cereals. Moreover, regarding legumes the roles of exogenous ALA through different modes in the induction of water stress tolerance has not yet been reported/explored. It was hypothesized that exogenous application of ALA through different modes may result in ameliorating the water stress induced adverse impacts with the aim for improvements growth and yield of two differentially water stress tolerant mungbean lines in relation with the plant water relations, photosynthetic pigments, lipid peroxidation and oxidative defense mechanism. The objectives of the study also include the selection of most effective level of ALA for the induction of water stress tolerance.

MATERIALS AND METHODS

Experimental conditions

Present experiment was conducted to assess the response of two differentially drought tolerant high yielding mung bean genotypes (16003 and 16004) to exogenously supplied ALA through different modes when grown under field water deficit conditions. The whole experiment was conducted in the Adaptive Research Complex located in Sheikhupura, Punjab Pakistan. The experiment was conducted from September –to December in 2020 and 2021 in two consecutive years. However, the data presented for different attributes in this study is given only for the single year due to similar findings. These selected mungbean lines are being cultivated by the farmers due to their high yielding potential. The seeds of these lines were obtained from the lentil section of Ayub Agriculture Research Institute (AARI). There were two levels of water stress i.e., normal irrigation (3 irrigation during growth period) and deficit irrigation (only one irrigation during the whole growth period only at the reproductive stage. The experiment was arranged in a split-split plot design with three replicates for each treatment. The experimental area was comprised two main plots corresponding to each irrigation level (normal irrigation and deficit irrigation). Each main plot was divided to two subplots each specific for mungbean genotype. Each subplot was further divided into three subplots corresponding to ALA mode of application (Foliar spray, seed priming and foliar + priming). In each sub-sub plot, there were 9 furrows of 6m length with a furrow-to-furrow distance of 60cm. A set of three furrows was specified for each specific level of ALA (0, 0.1 and 0.15mM of ALA). For seed priming treatment the seeds of both mungbean lines were primed separately with each specific level of ALA solution for 6h before sowing. After soaking the seeds were air-dried until the constant weight was achieved. Forty seeds were hand sown in each row with a 15cm distance. The sowing for soaked and non-soaked seeds was done at same time. Before sowing the soil was well prepared by ploughing when the soil was at the field capacity.

After 8 days of seed germination, thining was done to maintain 30cm plant to plant distance. During ploughing and land preparation the soil was supplied with adequate amount of fertilizer [N(160Kg/ha), P(80Kg/ha) and K(50Kg/ha)] as per the recommendations. Mungbean lines specified for foliar spray were sprayed with specific levels of ALA in evening for the maximum absorption of applied solution. Tween-20 (0.1%) was added to solutions prepared for each treatment prior to the foliar spray. Soil was covered with polythene sheet before the foliar application and then applied the ALA spray on each plant with the help of spray bottle for the accuracy of results. Fifty milli liter of solution of ALA of each treatment was applied to each specific row having 20 plants in each row. The foliar spray to plants specified for dual treatment (seed priming + foliar) were also sprayed at same time. After two weeks of foliar spray five plants were harvested from each treatment for the estimation of different growth, morphological and biochemical attributes. While the yield attributes were recorded at the maturity. The fresh leaf samples at the same time were collected and stored at -80^{o}C to be used further for different biochemical analysis.

The growth parameters measured were of the shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot length (SL), and roots length (RL). Biochemical analysis measured included the leaf photosynthetic pigments such as leaf chlorophyll *a* (Chl. *a*), chlorophyll *b* (Chl. *b*), total chlorophyll contents (T. Chl.) and chlorophyll *a/b* ratio (Chl. *a/b*), leaf proline content, glycine betaine (GB), total phenolics content (TPC), ascorbic acid (AsA) content, total flavonoids content (TFC), total soluble proteins (TSP), leaf hydrogen peroxide (H_2O_2) levels and leaf malondialdehyde contents (MDA) as well as the leaf antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). The plant samples specified for fresh biomasses were placed in an electric oven at 70 °C for 48 h to get the dry weight after measuring the fresh masses of roots and shoots.

Climatic conditions during experimentation

The averaged climatic conditions during the experimental period from September to December, during 2020 were as; photosynthetically active radiations (PAR) 972μmolm^{-2}s^{-1} maximum temperature 28.6±1.2°C, minimum temperature 14.75±1.3°C, relative humidity day 52.02±1.9%, relative humidity night 76.97±3.5% and 4.17±2.62 mm average rainfall pattern.

Soil texture and physico-chemical properties

The soil of the experimental site was sandy loam having available total N (0.73%) and organic matter (1.15%), and having P (8.6 ppm). The saturation percentage of the experimental soil was 34%. The average EC and pH of the soil was 2.53 ds.m-1 and 7.8, respectively. The soil solution has the soluble CO_3^{2-} (traces), SO_4^{-2} (1.98 meq L^{-1}), HCO_3^- (4.93 meqL^{-1}), $Ca^{2++}Mg^{2+}$ (14.3 meq L-1), Cl- (8.52 meq L^{-1}), Fe

(0.041meq L^{-1}), Na (2.98 meq L^{-1}) and SAR (0.086 meq L^{-1}). The soil's physical and chemical properties were assayed following *Davis & Freitas, 1970*.

Estimation of growth and morphological attributes

Carefully, one plant per replicate was uprooted in order to measure the fresh biomass of the root and shoot. Following washing, the root sample was blotted dry with paper to remove any remaining water. The fresh biomass of the shoot and root samples was then measured with an electric balance. Using a meter rod, the lengths of the shoot and root were measured simultaneously. The dry biomasses of the shoots and roots of these plants were then measured after being maintained at 65°C for 72 hours.

Determination of leaf photosynthetic pigments

The contents of Leaf Chl. a and Chl. b were estimated by using Arnon (1949) approach. An extract of 0.5g of fresh leaf was made in 10ml of acetone (80%). For five minutes, the extract was centrifuged at 10,000 ×g. At 480, 645, and 663 nm, the absorbance of the supernatant was measured using a spectrophotometer. Using the following formulas, the contents of Chl. a and Chl. b were determined:

Chl. *b* (measured in mg g^{-1} FW) = [22.9 (OD 645) -4.68 (OD 663)] x V/1000 x W

Chl. *a* (measured in mg g^{-1} FW) = [12.7 (OD 663) -2.69 (OD 645)] x V/1000 x W

V = volume of the leaf extract (measured in mL)

W = fresh weight of the leaf tissue (measured in g)

However, for the estimation of leaf carotenoid contents, the formula given by Kirk and Allen (1965) was used.

Carotenoids (measured in mg mL^{-1}) = A car/Em 100% × 1007

A Car (carotenoid) = (OD 480) + 0.114 (OD 663) − 0.638(OD 645)

Em (Emission) = Em 100% = 2500

Determination of leaf osmolytes content

Using *Grieve & Grattan's, 1983* methodology the GB content was determined. Homogenized the 0.5g dry leaf material using 10 ml of distilled water and kept it over-night at 4°C. Centrifuged the samples at 10,000 × *g* for 10 minutes. Then the 1 ml of the extract and 1 ml of 2N sulfuric acid was mixed properly and 0.2 ml of potassium tri-iodide solution (KI_3) was added to it. After cooling well, the mixture for 90 minutes in an ice bath, 2.8 ml of distilled water and 5 ml of 1-2 dichloroethane added to the solution. When the two layers formed then collect the lower layer by removing the upper layer. Measured the absorbance of lower layer at 365 nm spectrophotometrically. Laef proline content was determined following the method recommended by Bates *et al.* (1973). Fresh leaf 0.25g thawed in 10ml of 3% sulfosalicylic acid solution and filtered the mixture. Then, added 2ml of filtrate into the test tube and 2ml of acid ninhydrin along with 2ml of glacial acetic acid was added in it. For the preparation of acid ninhydrin: ninhydrin 1.26 g was mixed in in glacial acetic acid (30ml) and 6*M* ortho--phosphoric acid (20ml). Then the resultant mixture was incubated at 100°C for an hour. Cool down the solution and 2ml toluene was added to the mixture and vortexed well. Took the upper layer and measured the absorbance at 520 nm using the spectrophotometer.

Estimation of activities of enzymatic antioxidants and the contents of non-enzymatic antioxidants

Estimation of CAT, POD and SOD activities

For the estimation of the activities of antioxidant enzymes, TSP, and free amino acids the fresh leaf material (0.5g) was homogenized in 50mM potassium phosphate buffer having pH 7.8. The homogenize was centrifuged at 20,000 ×*g* at 4°C and the supernatant was stored at -20°C and later on used for the estimation of activities of antioxidant enzymes and TSP. The activities of CAT and POD were determined using the methods adopted by *Chance & Maehly, 1955*. For the estimation of CAT, the reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 59 mM H_2O_2, and 0.1 mL enzyme extract. The enzyme extract (100 µL) was mixed in the last to start the reaction. The change in absorbance was recorded at 240 nm for 120 s at intervals of 20 s. For the estimation of POD activity, the reaction mixture was prepared using 0.1 mL enzyme extract, 40 mM H_2O_2, 20 mM guaiacol and 50 mM phosphate buffer (pH 7.8). The change in the absorbance was measured at 470 nm for 120 s at intervals of 20 s.

The Giannopolitis & Ries, 1977 approach was used to measure the activity of SOD. A reaction mixture (1mL) including 50 µM NBT (NBT solution prepared in formamide), 13 mM methionine, 1.3 µM riboflavin, 75 nM EDTA, and 50 mM phosphate buffer (pH 7.8) was prepared using 50 µL of the enzyme extract. The reaction mixture was then placed within an aluminum foil and exposed to a 20 V bulb for 15 minutes. Before the reaction mixture was exposed to the light source, riboflavin was added. Every time, a blank sample was made without any extract added. Using a spectrophotometer, the absorbance of the reaction mixture was determined at 560 nm.

Estimation of leaf AsA content

According to the method given by *Mukherjee & Choudhuri, 1983* was used for the estimation of leaf AsA content. Fresh leaf material (0.25g) was ground in 10 ml of 6% TCA solution. After centrifugation the supernatant (4ml) was mixed with 2 ml of 2% dinitrophenyl hydrazine in prepared in 9 *N* H_2SO_4. One drop of 10% thiourea was added to the solution. The thiourea solution was prepared by mixing the 2g of thio-urea with 14 ml ethanol and 5 ml of distilled water. Incubated the mixture for 15 minutes in a water bath. After that, cooled the solution at room temperature and 5 ml of 80% H_2SO_4 added. The absorbance of the solution was read a using spectrophotometer at 530 nm.

Estimation of leaf TPC and TFC

Following the approach outlined by *Julkunen-Tiitto, 1985* was used to measure the seedling TPC. Fresh leaf 0.25_g mixed with 5ml of 80% acetone and then centrifuged at 10,000 × *g* for 10 minutes. Then, in a microfuge tube collected the resultant supernatant and stored at 4 °C. After it, 0.1_ml supernatant mixed with 2ml distilled water along with Folin-Ciocalteu's phenol reagent (1ml) and shake gently. Then to the above mixture 5ml of 20% Na_2CO_3 was added and the final volume was made up to 10ml by adding distilled water. The absorbance of the triturate was recorded at 750 nm by using spectrophotometer. The leaf TFC was determined spectrophotometry according to *Karadeniz et al., 2005*. Using a mortar and pestle, one gram of plant leaf material was taken and ground in 20 milliliters of 80% aqueous methanol. The filtrate was then obtained by filtering. Add 0.3 mL of 5% NaNO2 and 3 mL of distilled water to the filtrate (0.5 mL). The solution was allowed to stand at room temperature after thoroughly mixing. Following that, triturate and 0.6 mL of 10% AlCl3 were combined. 2 mL of 1M NaOH was also added after 6 minutes. Using distilled water, the solution's volume was kept at 10 mL. The final solution's absorbance was measured spectrophotometrically at 510 nm.

Estimation of TSP

For the determination of TSP content, the earlier obtained phosphate buffer solution was used as used earlier for the estimation of antioxidant enzymes. Supernatant (0.1ml) was reacted with 2mL of Bradford reagent and the absorbance was measured at 595 nm following the method given by *Bradford, 1976*.

Estimation of H_2O_2 and MDA contents

The levels of MDA represent the extent of lipid peroxidation (membrane damaging) under the conditions of stresses due to overly produced ROS. Using the approach outlined by *Cakmak & Horst, 1991*. Fresh leaf (0.25g) was ground in 1% TCA (3ml) at room temperature. At 5000 ×*g* centrifuged the extract for 15 minutes. Then 1ml of supernatant was mixed with 4_ml of 0.5% TBA prepared in 20% TCA solution. Incubate the solution at 95 ^{o}C for 50 minutes and the samples were then cooled at room temperature. The absorbance of the supernatant was measure at 532 and 600 nm by using the spectrophotometer. For the estimation of H_2O_2 content the method proposed by *Velikova et al., 2000* was used. The supernatant 0.5 mL was mixed with 1M KI, and incubated for 50min at 95°C, and absorbance was taken at 390 nm after cooling well.

Estimation of yield attributes

Two plants per replicate were harvested at maturity stage for determination of different yield attributes such as number of seed per pod, number of pods per plant, hundred grain weight (100GW) and total grain yield. The pods were collected manually from the plants and then dried in sunlight.

Statistical Analysis

The data was subjected to ANOVA for statistical analysis to find out the significant differences using CoStat CoHort software. In the studied attributes between treatments to determine the significant variations, the CoStat Computer Program (Monterey, CA, 93940 USA, PMB 320, Windows version 6.303) was used. The computer program XLSTAT (Addinsoft, Paris, France) was used to find out the correlations among the studied attributes.

RESULTS

Changes in growth attributes and oxidative stress markers

The growth of mungbean lines increased significantly when supplied with ALA levels (already screened in pilot experiment) when applied with different modes under water stress that presents a marked ameliorating responses of ALA against water stress (Fig. 1 and 2). The SFW, SDW, RFW, RDW, RL and SL significantly decreased with the imposition of water stress. Application of ALA through different modes showed significant increase in the values of studied growth parameters in both mungbean lines. Both of the levels i.e 0.1mM and 0.15mM of ALA showed non-significant difference with each but significantly higher values compared to non-treated ones. However, the mode of application of ALA showed significant variable response. All of these growth attributes showed significantly increased more in plants raised from seeds primed with different levels ALA compared to non-primed ones. Seed priming with different levels of ALA improved more the SFW, SDW, RFW, RDW, SL and RL as compared to plants grown with other application modes. However, the foliar application in combination with priming showed comparatively limited effects. SFW and SDW showed significant increase under foliar application of both levels only under water stress conditions. Both of the levels of foliar application of ALA significantly increased the RDW in both mungbean lines while RFW was significantly higher only in V2 line (16004) under both water stress and non-stress conditions. Foliar application of 0.15mM level significantly increased more the SL and RL of both mungbean lines under both water stressed and non-stressed condition than other treatments.

Imposition of water stress markedly increased the membrane permeability H_2O_2 levels and MDA content in both lines of mungbean as compared with plants grown under non-stressed conditions. Moreover, different ALA concentrations through different modes (0.1 and 0.15mM) when caused marked decreases in membrane permeability H_2O_2 levels and MDA content. However, the response of ALA application mode was observed significantly variable. For different modes of application as well as for different treatments the decrease was different. As compare to other application the priming mode of application showed higher values (Fig. 2).

Changes in leaf photosynthetic pigments

The plants raised under water stress conditions showed significant decrease in leaf Chl. *a*, Chl. *b* of both mungbean lines as compared to plants grown under non-stress conditions. Line16003 showed significantly more decrease than line 16004. Exogenous application of ALA when applied through different modes, significantly increased both Chl. *a* and Chl. *b* contents under both non-stressed and water stressed condition but this increase was more with seed priming than other treatments. This behavior was noted for both ALA levels. However, foliar application of ALA showed significant increase only in Chl. *b*. Chl. *a/b* ratio and T. Chl. were also recorded to be declined under water stress. However, ALA when applied as seed priming agent or through other modes, significantly increased both Chl. *a/b* and T. Chl. under both non-stressed and water stressed condition in both mungbean lines but more improvement was found due to seed priming than other treatments (Fig. 3).

Changes in osmolytes and non-enzymatic antioxidants

The changes in the levels of osmoprotectants such as proline and GB of both lines of mungbean in response to exogenous treatment of different concentrations (0.1 and 0.15 mM) of (ALA) and under water stress are presented in (Fig. 4) Imposition of water stress markedly increased the proline and GB in both lines of mungbean as compared with plants grown under non stressed conditions. Moreover, different ALA concentrations (0.1 and 0.15mM) when applied as seed priming agent or as foliar spray caused marked further increases in the studied proline content of both lines of mungbean as compared with their corresponding untreated ones. The similar results were found for GB but only when ALA was applied as priming agent and foliar application was found effective only for non-stressed plants of both lines. Combined application of priming and foliar level did not show any significantly increase in GB and proline in both lines and under both non stressed and water stressed condition. Combined application of ALA (0.1 and 0.15mM) as priming and foliar spray significantly increased the GB contents in non-stressed plants of line 16004.

Imposition of water stress caused significant decrease in leaf of both mungbean lines AsA contents relative to the non-stressed plants as shown in (Fig. 4). Exogenous application of ALA as seed priming or as foliar treatment with different concentrations (0.1 and 0.15 mM) improved the AsA contents of both mungbean lines both under non-stressed and water stressed conditions compared with those of untreated plants. ALA application as pre-sowing seed priming was found more effective approach in increasing leaf AsA contents in both mungbean lines grown under non stress and water stress conditions as compared with foliar application of ALA which showed significant increase in ascorbic acid content but only in non-stressed plants of line 16003.

Subjecting mungbean plants to water deficit stress caused significant decrease TPC of line 16003 relative to controls plant (Fig. 4). Exogenous application of ALA through different modes with different concentrations (0.1mM, 0.15mM) caused significant increase in leaf TPC contents in both lines of mungbean as compared with their corresponding untreated control plants. It is clear that both of the levels were highly equally effective as priming agent or when applied as foliar level as it caused the significant increases in TPC under both non stressed and water stressed plants of mungbean lines. However, the combined application of any level of ALA as seed priming or as foliar spray did not show any significant change in TPC contents both under non-stressed and water stressed conditions.

TSP and TFC contents

Under water stress conditions mungbean plants showed highly significant decrease in TSP and TFC content as compared to plants grown under non-stress conditions. Exogenous application of ALA with different concentrations (0.1mM, 0.15mM) caused significant increases in TSP and TFC content in both lines of mungbean as compared with their corresponding untreated controls (Fig. 5). However, the extent of increase in TSP and TFC content was different in different mode of applications. TSP and TFC content showed significant increase in plants raised from seeds primed with 0.1mM and 0.15mM levels of ALA as compared to non-treated ones. The other mode of application such as foliar application or foliar plus priming application of ALA did not show any significant increase from corresponding non-treated ones.

Changes in enzymatic antioxidants

Under water stress conditions mungbean plants showed highly significant decrease in CAT, SOD and POD activities as compared to plants grown under non-stress conditions. Exogenous application of ALA with different concentrations (0.1mM, 0.15mM) caused significant increases in enzymatic antioxidant activity in both lines of mungbean as compared with their corresponding untreated controls (Fig. 6.). However, the extent of increase in antioxidants was different in different mode of applications. All the three studied antioxidant enzymes showed significant increase in plants raised from seeds primed with 0.1mM and 0.15mM levels of ALA as compared to non-treated ones. The other mode of application such as foliar application or foliar plus priming application of ALA did not show any significant increase from corresponding non-treated ones.

Changes in yield attributes

Data presented in (Fig. 7) shows seed yield and yield attributes such as number of seed per pod, number of pods per plants, 100GW and TGY of two lines of mungbean decreased markedly by imposing water stress as compared to control plants. However, exogenous application of both levels of ALA (0.1mM, 0.15mM) applied either by seed priming or foliar spray caused significant increase in all parameters of yield components under non-stressed as well as under water stressed condition. On the other hand, combined application (priming +foliar) of any level of ALA did not show any significant change in this regard. Data show the superiority of line 16004 over line 16003 in yield and yield components.

Heat map

Data presented in (Fig. 8 & Fig. 9) regarding heatmap histogram correlation shows not only genotypic responses of mungbean genotypes to water deficit stress but also corresponds well the responses of genotype to exogenously applied different levels of ALA through different modes. The intensity of color of square in column and lines describes the intensity of positive and negative relationships among attributes. At x-axis categories in case of genotype V16003 4, 9 and 14 shows strong negative correlation (dark red colors) with maximum studied attributes except to H_2O_2, MDA and proline where a positive correlation was found without treatments and other showed less negative correlation. However, in case of genotype V16004 the intensity of negative impacts was less as present in brown color squares against x-axis attributes with categories 4, 14, 9 at y-axis (Fig 9). It shows a better performance of V 16004 in comparisons to V16003. Heatmap histogram based on intensities of colors (blue color) shows that a strong positive correlation can be observed of catagories 2 (0.1mM), 3 (0.15mM), 5 (0.1mM) and 6 (0.15mM) as seed priming treatment under non-stress and stress conditions respectively with studied attributes of both mungbean lines but comparatively more positive responses were found in genotype V16004. Catagories 7 and 8 corresponding to foliar treatments of 0.1 and 0.15mM ALA also showed positive influence but were less than the pre-sowing seed treatment. Foliar spray of ALA 0.1 and 0.15mM under stress and the combined treatments were not found so positively effective in both mungbean genotypes and showed an intermediate response as shown by light green squares in column and lines against the attributes x-axis.

DISCUSSION

One of environmental stresses responsible for decrease in plant growth and productivity is deficiency of fresh water for irrigation. Under deficiency irrigation plant undergo numerous metabolic modifications for their survival in present investigation, significant plant growth and yield of decrease in the studies of two lines of mungbean when grown under water stress. The results are in harmony with the studies of (*Sadak, 2016*; *Hossain et al., 2020*) who reported adverse impact deficit irrigation of different growth attributes of canola, wheat, quinoa, and moringa plants and narrated their decrease was associated with increased lipid peroxidation due to reactive oxygen species (ROS) over production and linked with. reduced cell elongation, cell turgor, cell volume and eventually the growth (*Banon et al., 2006*). Moreover, water stress also affects the plant–water relations, that causes osmotic stress that is responsible in also, imbibition of cell expansion and cell division responsible for continuity of growth of plants as a whole (*Alam et al., 2014*) Water stress leads to lowering of turgor pressure, which in turns suppresses cell expansion and cell growth (*Shehzad et al., 2022*). The reduction in plant height was found to be associated with a decrease in the cell enlargement under water stress as reported previously (*Wakchaure et al., 2023*).

Moreover, water deficit conditions directly suppress the development of optimal leaf area, which in turn causes decrease in photosynthesis and consequently the fresh, dry matter and yield (*Seleiman et al., 2021*). However, this reduction in growth and yield of mungbean plants due to water stress was less that were supplied with ALA through different modes and comparatively better in plants grown from seeds treated with ALA than foliar or combined application. It shows a clear role of ALA in plant stress tolerance that is due to the clear role in different physiological and biochemical molecules activities. Earlier studies have confirmed the promotive role of ALA on growth of plants grown under stress (*Youssef et al., 2021*) which referred this effect to the action of ALA as a growth regulator and reported as a protector against abiotic stress (*Ramadan et al., 2022*). Formerly, it is reported that exogenous application of ALA promoted root system in canola seedlings grown under stress and reported that (*Javeed et al., 2021*). ALA induced promotion of root system helpful in promoting the plant shoot system in growth by increasing the uptake of nutrients and water (*Ramadan et al., 2022*). This increase in plant growth could be attributed with the modulation in physiological processes such as nutrient uptake, photosynthesis and increasing antioxidant activities (*Youssef et al., 2021*). Moreover, in present it was found that ALA act as a potential modulator of plant growth and stress tolerance in a mode of application-dependent manner, where seed priming mode of application found superior one as compared to foliar or foliar plus priming treatment.

As exogenous applications of ALA through different modes improved the SFW, SDW, RFW and RDW and also the SL and RL of mungbean plants. Such type of growth improvement induced by ALA under stressful environment has already been studied in sorghum plants where ALA significantly improved the fresh and dry weight of shoot (*Youssef et al., 2021*). Moreover, our results also showed that exogenously applied different levels of ALA significantly improved the yield and yield attributes under water stress condition. Similar increase in wheat yield was observed in response to ALA (*Xiao et al., 2018*; *Elkelish et al., 2021*)

Alpha lipoic acid induced antioxidant application is known to accelerate the biomass and growth of plants by accelerating the cell enlargement, cell division and by improving membrane integrity resulting in reduced ion linkage (*Muscolo et al., 2014*; *Hassan et al., 2021*). In plants photosynthetic pigments play main role for harvesting light and then to transduce it into chemical energy. In this regard the pigments such as chlorophylls are indispensable to maintain optimum photosynthetic efficiency in plant (*Tabassum et al., 2016*). Under water stress conditions, the reduction in chlorophyll contents has been well reported depending on plant species specific (*Zargar, 2017*; *Shin et al., 2021*). but gravity and extent of water stress damage is water stress intensity specific (*Zargar, 2017*). and has been well reported in water stressed cotton (*Wang et al., 2016*), sunflower (*Hussain et al., 2018*) and pulses (*Tahir et al., 2019*) etc. The findings correspond well with that of earlier as reported by and that of presents finding where a (*Uddin et al., 2021*) decrease in mungbean Chl. contents under water deficient conditions has been found. The reason behind the decline in photosynthetic pigmentation under water stress might be due to stress induced stomatal closure leading to, modulation in gas exchange attributes as well as the reduced leaf area. (*Zargar et al., 2017*; *Liang et al., 2020*). Another factor responsible for decrease in photosynthetic pigments might be the reduced transcription of cab gene family which encodes the biosynthesis of chlorophyll pigment molecules (*Nikolaeva et al., 2010*) leading to decreased *de novo* synthesis of said pigments. Alternatively, destruction of the pigment protein complexes, might face destruction due to water stress (*Paim et al., 2020*) In addition, oxidative damage of chloroplast lipids and proteins also contribute in declining the chlorophyll *a, b* and carotenoids contents (*Liang et al., 2020*; *Uddin et al., 2021*).

In addition to stomatal factors non-stomatal constraints such as metabolic impairments are generally believed to be the main basis of reduced photosynthetic pigments in plants when grown under water deficient conditions (*Liang et al., 2020*). In the present study, exposure of mungbean plants to water deficient conditions noticeably altered the leaf Chl. contents. In this respect, it was observed that the Chl. *a*, Chl. *b*, Chl. *a/b* ratio and T. Chl. contents were significantly decreased in the water stressed mungbean plants compared to the well-watered counterparts that could be attributed in stressed activity of chlorophyllase (*Ali et al., 2018*) and oxidative damage (Figure, 2) which can not only damage the structure of chloroplast but also the cell membranes (*Ali et al., 2018*; *Hassan et al., 2020*). However, as another hand, plants exogenously supplied with (ALA showed less reduction in photosynthetic pigments including Chl. *a*, Chl. *b*, and Chl. *a/b* ratio and T. Chl. under water stressed conditions. Similar amelioration in stress induced damages to pigment by exogenously applied ALA has previously been reported, where ALA induced improvement in Chl. *a*, Chl. *b* and T. Chl. contents under stressful condition in wheat plants. Exogenously applied ALA mitigated the lipid peroxidation by stimulating the antioxidant systems in wheat and maize (*Gorcek & Erdal, 2015*; *Sezgin et al., 2019*; *Elkelish et al., 2021*). Chl. *a/b* ratio in our study in responding to ALA treatments in any mode under water stress condition significantly increase as compared to the untreated plants. This might be the fact that ALA as an efficient antioxidant has positive impacts on Chl. *a* and Chl. *b* by reducing the adverse impact of lipid peroxidation. Moreover, (*Elkelish et al., 2021*) reported that Chl. *a/b* ratio responded differentially to exogenous application of ALA under water stress conditions in wheat. Moreover, Chl. *a/b* ratio was observed to be unaffected in response to ALA application in wheat when grown under salt stress conditions as reported by (*Ramadan et al., 2022*). This indicated that effect of ALA species specific ALA application improves the ultra-structure of chloroplasts and preserving the chlorophyll and hence proving the promoting role in photosynthesis under water stress. (Youssef *et al*., 2021).

Alpha lipoic acid belongs to sulfur-containing biomolecules which are usually considered as very effective antioxidants and hence protect the plants when grown under abiotic stresses (*Gorcek & Erdal, 2015*). Exogenous application of ALA has been shown to stimulate not only the photosystem II but also regulate the gene expression of Rubisco and PEP carboxylase like certain carbon fixation enzymes in maize seedlings when grown under water stress conditions (*Sezgin et al., 2019*). All of these responses were coupled with a concurrent down-regulation in the chlorophyllase gene (Chlase) (*Sezgin et al., 2019*) which is further attributed to augment the N uptake, essential for biosynthesis of chlorophyll (*Sadak et al., 2020*). These findings can be correlated well with present results where exogenous application of ALA in any mode found effective in reducing adverse impact of water stress on leaf photosynthetic pigments.

Compatible solutes accumulation under water stress is a fundamental strategy for osmotic adjustment and they also play role in osmo-protection (*Majumdar et al., 2016*). Several evidences demonstrate that increased uptake of water and osmotic potential under osmotic stress (salinity or water stress) in plants is usually associated with accumulation of considerable concentrations of some organic molecules such as sugars and proline that improve plant water relations by performing role as osmo-regulators (*Alnusairi et al., 2021*; *Ibrahim et al., 2021*; *Nahhas et al., 2021*). Proline, GB, glycerol, sugars, mannitol and a number of low molecular weight metabolites are amassed in huge concentrations in plants in response to water stress to avoid the disintegration of proteins by working as osmoprotectants (*Shao et al., 2021*). Accumulation of proline decreases lipid oxidation and scavenge free radicals thus aids in maintaining the integrity of membrane (*Shinde et al., 2016*). Proline plays an important role during water stress, act as a signaling compound by regulating the function of mitochondria and affecting the proliferation of cell by means of activating particular genes which play important roles in stress recovery (*Solanki & Sarangi, 2014*). In present study imposition of water stress caused a marked increase in leaf proline and GB accumulation in both mungbean lines showing role in plant water relations by maintaining osmoregulation.

As regards exogenous application of ALA it has been found that it leads to improvement in leaf water potential through osmotic adjustment by accumulation of compatible solutes such as sugars and proline (*Terzi et al., 2018*). And it is well reported for osmolyte accumulation under other abiotic stresses too (*Gorcek & Erdal, 2015*; *Sezgin et al., 2019*; *Elkelish et al., 2021*; *Youssef et al., 2021*). In current study exogenously applied ALA further increased the accumulation of proline and GB in mungbean plants under water stress. These findings were in agreement with former reports on different crop plants (*Gorcek & Erdal, 2015*; *Terzi et al., 2018*; *Sezgin et al., 2019*; *Elkelish et al., 2021*; *Youssef et al., 2021*). Opposite to our findings in some species reduction in leaf proline content were recorded in ALA treated stressed wheat plants (Ramadan *et al*. 2022). As regards the effect of ALA treatment total soluble sugars it was found positive influence in maize plants (*Terzi et al., 2018*) In another study combined treatment of ALA and cysteine was found effective in improving leaf (RWC), total soluble sugars and leaf proline content of water stressed wheat (*Elkelish et al., 2021*). Finding shows strong correlation in compatible solute and plant water content (*Mohammad-khani & Heidari, 2008*). showing role of ALA is maintaining plant water relation through osmotic adjustment and similar has been found in present study

Regarding ROS production known as oxidative stress is a common phenomenon under the water stress. The ROS accumulation such as H_2O_2 leads to significant lipid peroxidation. (*Ali & Ashraf, 2011*). In the present study water stress increased leaf H_2O_2 content however, the exogenously applied ALA levels significantly decreased its level that is in agreement to previous studies (*Youssef et al., 2021*; *Ramadan et al., 2022*). Where ALA application reduced lipid peroxidation by reducing stress.

The interaction between increased ROS levels and ALA remains unknown under water stress, as limited literature is available regarding this treatment. To scavenge the ROS over production, plants have developed antioxidant defense mechanism based on enzymatic and non-enzymatic antioxidant compounds responsive for reduction of lipid peroxidation and antioxidant defense mechanism is cultivar /species specific (*Ali & Ashraf, 2011*) and is measured in terms of MDA contents in addition to non-enzymatic antioxidants and enzymatic antioxidants (*Ali et al., 2016*) It has been found that MDA content showing their better ROS scavenging potential that results in stable seedling growth (*Youssef et al., 2021*). In present study MDA content increased significantly in both mungbean lines under water stress. However, exogenously applied varying levels of ALA lowered the MDA level in contrast to an earlier study where MDA was reduced in stressed sorghum (*Youssef et al., 2021*) and wheat (*Ramadan et al., 2022*) after ALA treatment. Our results revealed that water stress induced substantial damage to cell membrane as depicted by increased H_2O_2 levels in mungbean. However, ALA induced decline in MDA and H_2O_2 is an indicative of better antioxidant system in the treated plants. These findings are in line with that of (*Gorcek & Erdal, 2015*). in wheat and yeast respectively. Several studies provided evidence that exogenous application of ALA restored the decreased levels of other antioxidants and reduced the damaging impact of oxidative stress in vivo under different physiological conditions (*Moini et al., 2002*), proving its role in protecting from stress induced lipid peroxidation (*Terzi et al., 2018*). It also plays a crucial role in the recycling of other oxidized radical scavengers such as ascorbate and glutathione by acting as free radical scavenger (*Navari-Izzo et al., 2002*). Thus, ALA can be considered as a potential candidate of antioxidants as having the ability to mitigate the oxidative damage induced by abiotic stresses (*Terzi et al., 2018*), such as water stress (*Sezgin et al., 2019*; *Elkelish et al., 2021*) and salt stress (*Youssef et al., 2021*). This positive role of ALA in improvement of antioxidative defense mechanism is negatively correlated with lipid peroxidation that is associated with the improved osmotic adjustment water relative less damage to photosynthesis pigments as a result better growth and biomass production under water stress.

However, regarding the antioxidative defense mechanism leaf AsA performs a crucial role in stressed plants by maintaining several metabolic processes serves as a reaction substrate within the enzymatic cycle as an electron donor to decrease the ROS accumulation (*Sadak et al., 2010*). Under stressful conditions it has been reported to rise or some time shows general reduction at the whole level of leaf in several species of plants (*Naz et al., 2022*). Diverse reports are available in literature, as in spinach its level was reduced but the reverse was true in case of soybean leaves (*Alizadeh et al., 2023*). In present study water stress significantly decreased the leaf AsA contents however, exogenously applied ALA improved its content in both lines of mungbean. Previously similar rise in AsA content was noted in ALA treated plants of wheat under osmotic stress (*Ramadan et al., 2022*) hence providing a close link between the attenuation of the oxidative damage and exogenous application of ALA mediated by non-enzymatic antioxidants.

Moreover, being strong antioxidants, TPC and TFC play well known important ecological and physiological role against different kind of stresses (*Wang et al., 2016*; *Li et al., 2019*). This accumulation of secondary metabolites mitigates the oxidative damages as one of the major strategies developed by the plants to induce the water stress tolerance (*Yadav et al., 2021*). TPC play defensive role to prevent plant cells from oxidative burst and thus protect plants from damage to RNA and DNA structures, lipids and proteins (*Ahmad et al., 2019*). In present study water stress caused significant reduction TPC and TFC in both mungbean lines similar as reported previously in grape wines (*Krol et al., 2014*) When significant reduction in TPC and TFC was found, this reduction could be attributes with the fact that in the stressed plants several changes occur in the carbon metabolism in order to attain the balance between the buildup of defensive secondary compounds and biomass production (*Akula & Ravishanka, 2011*) where the phenolic compounds are being used as sink for carbon under stress conditions hence are involved in plant tolerance (*Ferreyra et al., 2012*). In present study exogenously applied ALA significantly reduced the adverse impacts of water stress on leaf TPC in both lines of mungbean. The similar findings were recorded in wheat under water stress (*Elkelish et al., 2021*) and osmotic stress (*Ramadan et al., 2022*). This improvement in poly phenolics content is positively associated with better growth, better photosynthesis pigmentation and reduced lipid peroxidation shows the stress tolerance role of exogenously applied ALA in both mungbean cultivars when applied through different modes being better as seed treatment.

In present study activities of antioxidant enzymes such as CAT, SOD and POD decreased under stress. Previously, it was found that drought tolerant *Malus prunifolia* showed increase in the enzyme activities as compared to sensitive one (*Wang et al., 2012*; *Rai et al., 2021*). Similarly, it was found that water stress upsurges the activity of CAT (*Wang et al., 2012*) while a decrease was found CAT activity decreases under the water stress in rice seedlings (*Urmi et al., 2023*). Moreover, the antioxidant enzyme activities like SOD, POD, CAT were enhanced under the water stress in *Silybum marianum* (El-Sayed *et al*. 2019) and in sorghum (*Youssef et al., 2021*). However, exogenously applied different levels of ALA through different modes found effective in altering the CAT, SOD and POD activities in favor of better antioxidative defence mechanism. In earlier study it was reported that exogenous application of ALA elevated the antioxidant enzymatic activities such as SOD, CAT. MDHAR, GPX and GR under osmotic stress than to seedlings of maize not exposed to ALA. (*Terzi et al., 2018*). Similarly, it was found that exogenous treatments of ALA and cysteine improved the activities of enzymatic antioxidants in water stressed wheat plants (*Elkelish et al., 2021*). They reported that ALA applied increment in the activities of enzymatic antioxidants was associated with their stress tolerance in term of better growth and oxidative stress performance. Thus, it is confirmed from present findings as well as previous studies that ALA exogenous treatment improve antioxidative mechanism by acting enzyme activities that is specific to type of stress, ALA level and plant species (*Gorcek & Erdal, 2015*; *Elkelish et al., 2021*; *Youssef et al., 2021*)

It simply implies that under abiotic stresses ALA causes reduction in cytotoxicity of superoxide and H_2O_2 by modulating the activities of SOD and APX in barely and maize respectively (*Huang et al., 2019*; *Hassan et al., 2021*). It is further advocated that ALA contributes to instigate cellular redox management and ROS scavenging activities (*Gorcek, & Erdal, 2015*; *Turk et al., 2018*; *Terzi et al., 2018*; *Perveen et al., 2019*). Further investigations are needed to explore the mechanism of ALA in signaling pathway in the modification of antioxidative enzymes at organelles level in response to water stress conditions.

From the findings of present study it can be concluded that water stress tolerance induction in mungbean cultivar by exogenous use of ALA through different modes in term of better growth and yield is associated with better maintenance of water relation through osmotic adjustment, improvement in antioxidative defense mechanism with reduced lipid peroxidation, reduced membrane leakage, maintenance of better photosynthetic pigments in relation with reduced lipid peroxidation and better antioxidative defense mechanism. Among different modes of application seed priming treatment was found better than other treatments

However, variable limited reports in literature pointed out its specie and dose dependent behavior. Therefore, similar studies on other crops are needed in future to find out its exact stress tolerance mechanism. Additionally, there is lack of information about pathways modulation associated with ALA induced stress tolerance. Therefore, such studies should be the future focus of plant researchers working in this particular area of research. Thus, comprehensive genetic in relation with biochemical studies are needed to uncover mechanism of ALA in plants grown under water stress conditions.

CONCLUSIONS

We can conclude, from the results of this study that ALA can induce tolerance to water stress in mungbean plants. Data shows that against water stress, ALA has many protective aspects through enhancing plant growth, pigmentation, enzymatic as well as non-enzymatic antioxidant and yield and reducing the oxidative damage. The better growth and yield of mungbean plants grown from seeds treated as priming with ALA under water stress condition. Thus, it can be recommended that ALA as seed priming is more promising in combating the adverse effect of water stress, which will be surely helpful for more uniform and better crop stands and will lead to better production under water stress condition.

DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article or can be requested to the corresponding author.

Abbreviations

RFW= root fresh weight; **RDW**= root dry weight; **SFW**= shoot fresh weight; **SDW**= shoot dry weight; **SL**= shoot length; **RL**= root length; **Chl. *a***= chlorophyll *a*; **Chl. *b***= chlorophyll *b*; **TSP**= total soluble protein; **MDA**= malondialdehyde; **H_2O_2=** hydrogen peroxide; **SOD=** superoxide dismutase; **POD=** peroxide dismutase; **CAT=** catalase; **TFC=** total flavonoid content; **TPC=** Total phenolic content; **GB=** glycine betaine; **AsA=** ascorbic acids

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Author Contribution

The work presented in this manuscript is part of the PhD studies of Miss Naima Hafeez Mian and she conducted the experiment and lab analysis. The research was planned by Dr. Muhammad Azeem. The work was done under the guidance of Dr. Muhammad Azeem, Dr. Qasim Ali and Dr. Sohail Akram guided laboratory analysis and in the software used for statistical analysis.

Conflict of interest

Author have no conflict of interest

REFERENCES

Abd-Elhamid EM, Sadak MS, Tawfik MM. 2014. Alleviation of adverse effects of salt stress in wheat cultivars by foliar treatment with antioxidant 2- changes in some biochemical aspects, lipid peroxidation, antioxidant enzymes and amino acid contents. *Journal of Agricultural Science* **5(13)**: 1269-1280. http://dx.doi.org/10.4236/as.2014.513135

Ahmad R, Hussain S, Anjum MA, Khalid MF, Saqib M, Zakir I, Ahmad S. 2019. Oxidative stress and antioxidant defense mechanisms in plants under salt stress. *Plant Abiotic Stress Tolerance: Agronomic, Molecular and Biotechnological Approaches* 191-205. http://doi.org/10.1007/978-3-030-06118-0_8

Akula R, Ravishankar GA. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling and Behavior* **6(11)**: 1720-1731. https://doi.org/10.4161/psb.6.11.17613

Alam MM, Nahar K, Hasanuzzaman M, Fujita M. 2014. Exogenous jasmonic acid modulates the physiology, antioxidant defense and glyoxalase systems in imparting drought stress tolerance in different Brassica species. *Plant Biotechnology Reports* **8**: 279-293. http://doi.org/10.1007/s11816-014-0321-8

Alban FT, Gyamfi D, van-Golen RF, Heger M. 2018. Reactive oxygen and nitrogen species and liver ischemia-reperfusion injury: role of lipoic acid. In: *The Liver* (pp. 109-119). Academic Press.

Ali Q, Ashraf M. 2011. Induction of drought tolerance in maize (*Zea mays* L.) due to exogenous application of trehalose: growth, photosynthesis, water relations and oxidative defence mechanism. *Journal of Agronomy and Crop Science* **197(4)**: 258-271. https://doi.org/10.1111/j.1439-037X.2010.00463.x

Ali Q, Haider MZ, Iftikhar W, Jamil S, Javed MT, Noman A, Perveen R. 2016. Drought tolerance potential of *Vigna mungo* L. lines as deciphered by modulated growth, antioxidant defense, and nutrient acquisition patterns. *Brazilian Journal of Botany* **39**: 801-812. http:doi.org/10.1007/s40415-016-0282-y

Ali Q, Javed MT, Noman A, Haider MZ, Waseem M, Iqbal N, Perveen R. 2018. Assessment of drought tolerance in mung bean cultivars/lines as depicted by the activities of germination enzymes, seedling's antioxidative potential and nutrient acquisition. *Archives of Agronomy and Soil Science* **64(1):** 84-102. https://doi.org/10.1080/03650340.2017.1335393

Alizadeh R, Kumleh HH, Rezadoost MH. 2023. The simultaneous activity of cytosolic and mitochondrial antioxidant mechanisms in neutralizing the effect of drought stress in soybean. *Plant Physiology Reports* **28(1):** 78-91. http://doi.org/10.1007/s40502-022-00704-6

Allito BB, Nana EM, Alemneh AA. 2015. Rhizobia strain and legume genome interaction effects on nitrogen fixation and yield of grain legume: a review. *Molecular Soil Biology* **6**: 1-12. https://doi.org/10.5376/msb.2015.06.0004

Alnusairi GS, Mazrou YS, Qari SH, Elkelish AA, Soliman MH, Eweis M, El-Nahhas N. 2021. Exogenous nitric oxide reinforces photosynthetic efficiency, osmolyte, mineral uptake, antioxidant, expression of stress-responsive genes and ameliorates the effects of salinity stress in wheat. *Plants* **10(8)**: 1693. https://doi.org/10.3390/plants10081693

Arnoldi A, Zanoni C, Lammi C, Boschin G. 2014. The role of grain legumes in the prevention of hypercholesterolemia and hypertension. *Critical Reviews in Plant Sciences* **34(1-3)**: 144-168. https://doi.org/10.1080/07352689.2014.897908

Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiology* **24**: 1. http://doi.org/ https://doi.org/10.1104%2Fpp.24.1.1

Bangar P, Chaudhury A, Tiwari B, Kumar S, Kumari R, Bhat KV. 2019. Morphophysiological and biochemical response of mungbean [*Vigna radiata* (L.) Wilczek] varieties at different developmental stages under drought stress. *Turkish Journal of Biology* **43:** 58-69. https://doi.org/10.3906%2Fbiy-1801-64

Banon S, Ochoa J, Franco JA, Alarcon J, Sánchez-Blanco MJ. 2006. Hardening of oleander seedlings by deficit irrigation and low air humidity. *Environmental and Experimental Botany* **56(1)**: 36-43. https://doi.org/10.1016/j.envexpbot.2004.12.004

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil* **39**: 205-207. http://doi.org/ 10.1007/bf00018060

Boelt B, Julier B, Karagic D, Hampton J. 2014. Legume seed production meeting market requirements and economic impacts. *Critical Reviews in Plant Sciences* **34(1-3)**: 412-427. https://doi.org/10.1080/07352689.2014.898477

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72:** 248-254. doi: 10.1006/abio.1976.9999

Cakmak I, Horst WJ. 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Plant Physiology* **83(3)**: 463-468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x

Çelik S, Ozkaya A. 2002. Effects of intraperitoneally administered lipoic acid, vitamin E, and linalool on the level of total lipid and fatty acids in guinea pig brain with oxidative stress induced by $H_2 O_2$. *Journal of Biochemistry and Molecular Biology Reports* **35 (6)**: 547-552. doi: 10.5483/bmbrep.2002.35.6.547

Chance B, Maehly AC. 1955. Assay of catalases and peroxidases. *Methods in Enzymology* **2**: 764-775. https://doi.org/10.1016/S0076-6879(55)02300-8

Chen LG, Gottschalck J, Hartman A, Miskus D, Tinker R, Artusa A. 2019. Flash drought characteristics based on US drought monitor. *Journal of Atmosphere* **10(9)**: 498. https://doi.org/10.3390/atmos10090498

Ebert AW. 2014. Potential of underutilized traditional vegetables and legume crops to contribute to food and nutritional security, income and more sustainable production systems. *Sustainability* **6 (1)**: 319-335. https://doi.org/10.3390/su6010319

Elkelish A, El-Mogy MM, Niedbała G, Piekutowska M, Atia MA, Hamada MM, Ibrahim MF. 2021. Roles of exogenous α-lipoic ccid and cysteine in Mitigation of drought stress and restoration of grain quality in wheat. *Plants* **10(11)**: 2318. https://doi.org/10.3390/plants10112318

El-Sayed AS, Mohamed NZ, Safan S, Yassin MA, Shaban L, Shindia AA, Sitohy MZ. 2019. Restoring the taxol biosynthetic machinery of *Aspergillus terreus* by *Podocarpus gracilior Pilger microbiome*, with retrieving the ribosome biogenesis proteins of WD40 superfamily. *Scientific Reports* **9(1)**: 11534. https://doi.org/10.1038/s41598-019-47816-y

Favero VO, Carvalho RH, Motta VM, Leite ABC, Coelho MRR, Xavier GR, Urquiaga S. 2021. *Bradyrhizobium* as the only rhizobial inhabitant of mung bean (*Vigna radiata*) nodules in tropical soils: a strategy based on microbiome for improving biological nitrogen fixation using bio-products. *Frontiers of Plant Science* **11**: 602645. https://doi.org/10.3389/fpls.2020.602645

Ferreyra ML, Rius SP, Casati P. 2012. Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Frontiers of Plant Science* **3**: 222. https://doi.org/10.3389/fpls.2012.00222

Fogacci F, Rizzo M, Krogager C, Kennedy C, Georges CM. 2020. Safety evaluation of α-lipoic acid supplementation: a systematic review and meta-analysis of randomized placebo-controlled clinical studies. *Antioxidants (Basel)* **9(10)**: 1-34. https://doi.org/10.3390/antiox9101011

Foyer CH, Lam HM, Nguyen HT, Siddique KH, Varshney RK, Colmer TD, Considine MJ. 2016. Neglecting legumes has compromised human health and sustainable food production. *Nature Plants* **2(8)**: 1-10. https://doi.org/10.1038/nplants.2016.112.

Giannopolitis CN, Ries SK. 1977. Superoxide dismutases: I Occurrence in higher plants. *Plant Physiology* **59(2)**: 309-314. https://doi.org/10.1104/pp.59.2.309

Gorcek Z, Erdal S. 2015. Lipoic acid mitigates oxidative stress and recovers metabolic distortions in salt-stressed wheat seedlings by modulating ion homeostasis, the osmo-regulator level and antioxidant system. *Journal of the Science of Food and Agriculture* **95(14)**: 2811-2817. https://doi.org/10.1002/jsfa.7020

Grieve CM, Grattan SR. 1983. Rapid assay for determination of water-soluble quaternary ammonium compounds. *Plant and Soil* **70**: 303-307. http://doi.org/ 10.1007/BF02374789

Guo Y, Huang E, Yuan C, Zhang L, Yousef AE. 2012. Isolation of a *Paenibacillus sp.* strain and structural elucidation of its broad-spectrum lipopeptide antibiotic. *Applied and Environmental Microbio*logy **78(9)**: 3156-3165. https://doi.org/10.1128/AEM.07782-11

Hassan A, Amjad SF, Saleem MH, Yasmin H, Imran M, Riaz M, Alyemeni MN. 2021. Foliar application of ascorbic acid enhances salinity stress tolerance in barley (*Hordeum vulgare* L.) through modulation of morpho-physio-biochemical attributes, ions uptake, osmo-protectants and stress response genes expression. *Saudi Journal of Biological Sciences* **28(8)**: 4276-4290. https://doi.org/10.1016/j.sjbs.2021.03.045

Hassan N, Ebeed H, Aljaaran, A. 2020. Exogenous application of spermine and putrescine mitigate adversities of drought stress in wheat by protecting membranes and chloroplast ultra-structure. *Physiology and Molecular Biology of Plants* **26(2)**: 233-245. http://doi.org/10.1007/s12298-019-00744-7

Hossain MZ, Sikder S, Husna A, Sultana S, Akhter S, Alim A, Joardar JC. 2020. Influence of water stress on morphology, physiology and yield contributing characteristics of rice. *SAARC* J*ournal of Agricultural Science* **18(1)**: 61-71. https://doi.org/10.3329/sja.v18i1.48382

Huang B, Chen, YE, Zhao YQ, Ding CB, Liao JQ, Hu C, Yuan M. 2019. Exogenous melatonin alleviates oxidative damages and protects photosystem II in maize seedlings under drought stress. *Frontiers of Plant Science* **10**: 677. https://doi.org/10.3389/fpls.2019.00677

Huang T, Huang K. 2004. The biomedical function of α-lipoic acid. *Journal of Medical Biochemistry* **24(1):** 58-60.

Hussain M, Farooq S, Hasan W, Ul-Allah S, Tanveer M, Farooq M, Nawaz A. 2018. Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agricultural Water Management* **201**: 152-166. https://doi.org/10.1016/j.agwat.2018.01.028

Ibrahim MFM, Ibrahim HA, Abd El-Gawad HG. 2021. Folic acid as a protective agent in snap bean plants under water deficit conditions. *Journal of Horticultural Science and Biotechnology* **96(1)**: 94-109. https://doi.org/10.1080/14620316.2020.1793691

Javeed HMR, Ali M, Skalicky M, Nawaz F, Qamar R, Rehman AU, El-Sabagh A. 2021. Lipoic acid combined with melatonin mitigates oxidative stress and promotes root formation and growth in salt-stressed canola seedlings (*Brassica napus* L.). *Molecules* **26(11)**: 3147. https://doi.org/10.3390/molecules26113147

Julkunen-Tiitto, R. 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry* **33(2)**: 213-217. https://doi.org/10.1021/jf00062a013

Karadeniz F, Burdurlu HS, Koca N, Soyer Y. 2005. Antioxidant activity of selected fruits and vegetables grown in Turkey. *Turkish Journal of Agriculture and Forestry* **29:** 297-303.

Khalid MF, Hussain S, Ahmad S, Ejaz S, Zakir I, Ali MA, Anjum MA. 2019. Impacts of abiotic stresses on growth and development of plants. In *Plant Tolerance to Environmental Stress* CRC Press, 1-8. http://dx.doi.org/10.1201/9780203705315-1

Krol A, Amarowicz R, Weidner S. 2014. Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of long-term drought stress. *Acta Physiologiae Plantarum* **36**: 1491-1499. http://doi.org/10.1007/s11738-014-1526-8

Li N, Han X, Feng D, Yuan D, Huang LJ. 2019. Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense: do we understand what they are whispering?. *International Journal of Molecular Sciences* **20(3)**: 671. https://doi.org/10.3390/ijms20030671

Liang X, Zhang T, Lu X, Ellsworth DS, Bassirirad H, You C, Ye Q. 2020. Global response patterns of plant photosynthesis to nitrogen addition: A meta-analysis. *Global Change Biology* **26(6)**: 3585-3600. https://doi.org/10.1111/gcb.15071

Lindstrom K, Mousavi SA. 2020. Effectiveness of nitrogen fixation in rhizobia. *Microbial Biotechnology* **13(5)**: 1314-1335. https://doi.org10.1111/1751-7915.13517

Liu F, Andersen MN, Jacobsen SE, Jensen CR. 2005. Stomatal control and water use efficiency of soybean (*Glycine max* L.) during progressive soil drying. *Environmental and Experimental Botany* **54(1)**: 33-40. https://doi.org/10.1016/j.envexpbot.2004.05.002

Majumdar R, Barchi B, Turlapati S A, Gagne M, Minocha R, Long S, Minocha SC. 2016. Glutamate ornithine, arginine, proline, and polyamine metabolic interactions: the pathway is regulated at the post-transcriptional level. *Frontiers of Plant Science* **7**: 1-17. https://doi.org/10.3389/fpls.2016.00078

Maksup S, Roytrakul S, Supaibulwatana K. 2012. Physiological and comparative proteomic analyses of Thai jasmine rice and two check cultivars in response to drought stress. *Journal of Plant Interactions* 9**(1)**: 43-55. https://doi.org/10.1080/17429145.2012.752042

Mian HN, Mahmood S, Akram S, Ali Q, Azeem M. 2021. Evaluation of drought tolerance potential in mungbean *Vigna radiata* (L.) R. Wilczek germ plasm resources: growth pigmentation and oxidative defense mechanism. *Fresenius Environmental Bulletin* **30(8)**:10313-10325.

Mohammad-khani N, Heidari R. 2008. Water stress induced by polyethylene glycol 6000 and sodium. *Pakistan Journal of Biological Sciences* **2(1)**: 92-97. https://doi.org/10.3923/pjbs.2008.92.97

Moini H, Packer L, Saris NEL. 2002. Antioxidant and prooxidant activities of α-lipoic acid and dihydrolipoic acid. *Toxicology and Applied Pharmacology* **182(1)**: 84-90. https://doi.org/10.1006/taap.2002.9437

Mujahid M, Akram MZ, Nazeer S. 2022. Evaluation of mung bean (*Vigna radiata*) under drought stress by foliar application of salicylic acid. *Journal of Agriculture, Food, Environment and Animal Sciences* **3(1)**: 1-14. https://www.jafeas.com/index.php/j1/article/view/29

Mukherjee SP, Choudhuri MA. 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiologia Plantarum* **58(2)**: 166-170. https://doi.org/10.1111/j.1399-3054.1983.tb04162.x

Muscolo A, Sidari M, Anastasi U, Santonoceto C, Maggio A. 2014. Effect of PEG-induced drought stress on seed germination of four lentil genotypes. *Plant Interaction* **9(1)**: 354-363. https://doi.org/10.1080/17429145.2013.835880

Nahhas NE, Abdelaal KA, Al-Kahtani MD, Husnain L, Al-Gwaiz H I, Hafez YM. 2021. Biochar and jasmonic acid application attenuate antioxidative systems and improves growth, physiology, nutrient uptake and productivity of faba bean (*Vicia faba* L.) irrigated with saline water. *Plant Physiology and Biochemistry* **166**: 807–817. https://doi.org/10.1016/j.plaphy.2021.06.033

Nair RM, Pandey AK, War AR, Hanumantharao B, Shwe T, Alam AKMM, Schafleitner R. 2019b. Biotic and abiotic constraints in mungbean production progress in genetic improvement. *Frontiers of Plant Science* **10**: 1340. https://doi.org/10.3389/fpls.2019.01340

Nair RM, Thavarajah P, Giri RR, Ledesma D, Yang RY, Hanson P, Hughes JDA. 2015a. Mineral and phenolic concentrations of mungbean [*Vigna radiata* (L.) R. Wilczek var. radiata] grown in semi-arid tropical India. *Journal of Food Composition and Analysis* **39**: 23-32. https://doi.org/10.1016/j.jfca.2014.10.009

Navari-Izzo F, Quartacci M F, Sgherri C. 2002. Lipoic acid: a unique antioxidant in the detoxification of activated oxygen species. *Plant Physiology and Biochemistry* **40(6-8)**: 463-470. https://doi.org/10.1016/S0981-9428(02)01407-9

Naz H, Akram NA, Ashraf M, Hefft DI, Jan BL. 2022. Leaf extract of neem (*Azadirachta indica*) alleviates adverse effects of drought in quinoa (*Chenopodium quinoa* Willd.) plants through alterations in biochemical attributes and antioxidants. *Saudi Journal of Biological Sciences* **29(3):** 1367-1374. https://doi.org/10.1016/j.sjbs.2022.01.038

Nikolaeva MK, Maevskaya SN, Shugaev AG, Bukhov NG. 2010. Effect of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity. *Russian Journal of Plant Physiology* **57**: 87-95. http://doi.org/10.1134/S1021443710010127

Paim BT, Crizel RL, Tatiane SJ, Rodrigues VR, Rombaldi CV, Galli V. 2020. Mild drought stress has potential to improve lettuce yield and quality. *Scientia Horticulturae* **272**: 1-7. https://doi.org/10.1016/j.scienta.2020.109578

Perez-Lopez U, Robredo A, Lacuesta M, Sgherri C, Mena-Petite A, Navari-Izzo F, Munoz-Rueda A. 2010. Lipoic acid and redox status in barley plants subjected to salinity and elevated CO_2. *Physiology Plantarum* **139(3)**: 256-268. https://doi.org/10.1111/j.1399-3054.2010.01361.x

Perveen S, Iqbal M, Saeed M, Iqbal N, Zafar S, Mumtaz T. 2019. Cysteine-induced alterations in physicochemical parameters of oat (*Avena sativa* L. var. Scott and F-411) under drought stress. *Biologia Futura* **70**: 16-24. http://doi.org/0.1556/019.70.2019.03

Pratap A, Douglas C, Prajapati U, Kumari G, War AR, Tomar R, Dubey S. 2020. Breeding progress and future challenges: biotic stresses. *The Mungbean Genome* **53**: 55-80. http://doi.org/ 10.1007/978-3-030-20008-4_5

Rai N, Morales LO, Aphalo PJ. 2021. Perception of solar UV radiation by plants: photoreceptors and mechanisms. *Plant Physiology* **186(3)**: 1382-1396. https://doi.org/10.1093/plphys/kiab162

Ramadan KM, Alharbi MM, Alenzi AM, El-Beltagi HS, Darwish DBE, Aldaej MI, Ibrahim MF. 2022. Alpha lipoic acid as a protective mediator for regulating the defensive responses of wheat plants against sodic alkaline stress: physiological, biochemical and molecular aspects. *Plants* **11(6)**: 787. https://doi.org/10.3390/plants11060787

Reed LJ, DeBusk BG, Gunsalus IC, Hornberger JCS. 1951. Crystalline α-lipoic acid: a catalytic agent associated with pyruvate dehydrogenase. *Science* **114(2952)**: 93-94. https://doi.org/10.1126/science.114.2952.93

Sadak MS. 2016. Mitigation of drought stress on fenugreek plant by foliar application of trehalose. *International Journal of Chemistry and Technology* **9(2)**: 147-155.

Sadak MS, Abdalla AM, Abd-Elhamid EM, Ezzo MI. 2020. Role of melatonin in improving growth, yield quantity and quality of *Moringa oleifera* L. plant under drought stress. *Bulletin of the National Research Centre* **44(1)**: 1-13. http://doi.org/10.1186/s42269-020-0275-7

Sadak MS, Abdelhamid MT, El-Saady KM. 2010. Physiological responses of faba bean plant to ascorbic acid grown under salinity stress. *Egyptian Journal of Agronomy* **32(1)**: 89-106.

Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Battaglia ML. 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* **10(2)**: 259. https://doi.org/10.3390/plants10020259

Sezgin A, Altuntas C, Demiralay M, Cinemre S, Terzi R. 2019. Exogenous alpha lipoic acid can stimulate photosystem II activity and the gene expressions of carbon fixation and chlorophyll metabolism enzymes in maize seedlings under drought. *Journal of Plant Physiology* **232**: 65-73. https://doi.org/10.1016/j.jplph.2018.11.026

Shao C, Shen L, Qiu C, Wang Y, Qian Y, Chen J, Peng C. 2021. Characterizing the impact of high temperature during grain filling on phytohormone levels, enzyme activity and metabolic profiles of an early indica rice variety. *Plant Biology* **23(5):** 806-818. https://doi.org/10.1111/plb.13253

Shehzad F, Ali Q, Ali S, Al-Misned FA, Maqbool S. 2022. Fertigation with Zn-Lysine confers better photosynthetic efficiency and yield in water stressed maize: water Relations, antioxidative defense mechanism and nutrient acquisition. *Plants* **11(3):** 404. https://doi.org/10.3390/plants11030404

Shin YK, Bhandari SR, Jo J S, Song JW, Lee JG. 2021. Effect of drought stress on chlorophyll fluorescence parameters, phytochemical contents, and antioxidant activities in lettuce seedlings. *Journal of Horticulture* **7(8)**: 238. https://doi.org/10.3390/horticulturae7080238.

Shinde S, Villamor JG, Lin W, Sharma S, Verslues PE. 2016. Proline coordination with fatty acid synthesis and redox metabolism of chloroplast and mitochondria. *Plant Physiology* **172(2):** 1074-1088. https://doi.org/10.1104/pp.16.01097

Singh CM, Singh AK, Mishra SB, Pandey A. 2016. Generation mean analysis to estimate the genetic parameters for yield improvement and inheritance of seed colour and lusture in mungbean [*Vigna radiata* (L.) Wilczek]. *Legume Research An International Journal* **39**: 1-18. http://doi.org/ http://dx.doi.org/10.18805/lr.v0iOF.10762

Singh P, Pandey B, Pratap A, Gyaneshwari U, Nair RM, Mishra AK, Singh CM. 2022. Genetic and genomics resources of cross-species vigna gene pools for improving biotic stress resistance in mungbean (*Vigna radiata* L. Wilczek). *Agronomy* **12(12):** 3000. https://doi.org/10.3390/agronomy12123000

Solanki JK, Sarangi SK. 2014. Effect of drought stress on proline accumulation in peanut genotypes. *International Journal of Advanced Research and Publications* **2(10)**: 301-309.

Tabassum MA, Zhu GL, Hafeez A, Wahid MA, Shaban M, Li Y. 2016. Influence of leaf vein density and thickness on hydraulic conductance and photosynthesis in rice (*Oryza sativa* L.) during water stress. *Science Reports* **6(1)**: 36894. https://doi.org/10.1038/srep36894

Tahir A, Qadir M, Saif R, Sattar S, Tahir S. 2019. Morphological and yield response of pulses against drought stress: A review. *Turkish Journal of Agriculture-Food Science and Technology* **7(2)**: 202-208. https://doi.org/10.24925/turjaf.v7i2.202-208.2178

Terzi R, Saruhan GN, Guven FG, Kadioglu A. 2018. Alpha lipoic acid treatment induces the antioxidant system and ameliorates lipid peroxidation in maize seedlings under osmotic stress. *Archives of Biological Sciences* **70(3)**: 503-511. https://doi.org/10.2298/ABS171218011T

Turk H, Erdal S, Karayel U, Dumlupinar R. 2018. Attenuation of lead toxicity by promotion of tolerance mechanism in wheat roots by lipoic acid. *Cereal Research Communications* **46**: 424-435. http://doi.org/ 10.1556/0806.46.2018.020

Uddin S, Ullah S, Nafees M. 2021. Effect of seed priming on growth and performance of *Vigna radiata* L. under induced drought stress. *Journal of Agriculture and Food Research* **4**: 100140. https://doi.org/10.1016/j.jafr.2021.100140

United Nations. World population prospects: Department of Economic and Social Affairs, Population Division. *United Nations.* 2019. *Online Edition*, Revision 1.

Uppalwar SV, Garg V, Dutt R. 2021. Seeds of mung bean (*Vigna radiata* (L.) R. wilczek): taxonomy, phytochemistry, medicinal uses and pharmacology. *Current Bioactive Compounds* **17(3)**: 220-233. https://doi.org/10.2174/1573407216999200529114608.

Urmi TA, Islam MM, Zumur KN, Abedin MA, Haque MM, Siddiqui MH, Hoque MA. 2023. Combined effect of salicylic acid and proline mitigates drought stress in rice (*Oryza sativa* L.) through the modulation of physiological attributes and antioxidant enzymes. *Antioxidants* **12(7):** 1438. https://doi.org/10.3390/antiox12071438

Velikova V, Yordanov I, Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Science* **151(1)**: 59-66. https://doi.org/10.1016/S0168-9452(99)00197-1

Wakchaure GC, Minhas PS, Kumar S, Khapte PS, Dalvi SG, Rane J, Reddy KS. 2023. Pod quality, yields responses and water productivity of okra (*Abelmoschus esculentus* L.) as affected by plant growth regulators and deficit irrigation. *Agricultural Water Management* **282:** 108267. https://doi.org/10.1016/j.agwat.2023.108267

Wang S, Liang D, Li C, Hao Y, Ma F, Shu H. 2012. Influence of drought stress on the cellular ultrastructure and antioxidant system in leaves of drought-tolerant and drought-sensitive apple rootstocks. *Plant Physiology and Biochemistry* **51:** 81-89. https://doi.org/10.1016/j.plaphy.2011.10.014

Wang X, Li Q, Yuan W, Cao Z, Qi B, Kumar S, Qian W. 2016. The cytosolic Fe-S cluster assembly component MET18 is required for the full enzymatic activity of ROS1 in active DNA demethylation. *Scientific Reports* **6(1)**: 26443. https://doi.org/10.1038/srep26443

Xiao R, Wang X, Jiang L, Tang H. 2018. Research and application of lipoic acid in plants. In *IOP Conference Series: Environmental Earth Sciences* **108**: 1-5. http://doi.org/ 0.1088/1755-1315/108/4/042100

Yadav B, Jogawat A, Rahman MS, Narayan OP. 2021. Secondary metabolites in the drought stress tolerance of crop plants: A review. *Gene Reports* **23:** 101040. https://doi.org/10.1016/j.genrep.2021.101040

Youssef M H, Raafat A, El-Yazied A A, Selim S, Azab E, Khojah E. 2021. Ibrahim M F Exogenous application of alpha-Lipoic acid mitigates salt-induced oxidative damage in sorghum plants through regulation growth, leaf pigments, ionic homeostasis, antioxidant enzymes, and expression of salt stress responsive genes. *Plants* **10(11)**: 2519. https://doi.org/10.3390/plants10112519

Zargar SM, Gupta N, Nazir M, Mahajan R, Malik FA, Sofi NR, Salgotra RK. 2017. Impact of drought on photosynthesis: Molecular perspective. *Plant Gene* **11**: 154-159. https://doi.org/10.1016/j.plgene.2017.04.003

Figure 1

SFW, SDW, RFW, RDW of differentially drought tolerant mungbean genotypes fertigated with different levels of ALA through different modes when grown under normal irrigation and deficit irrigation.

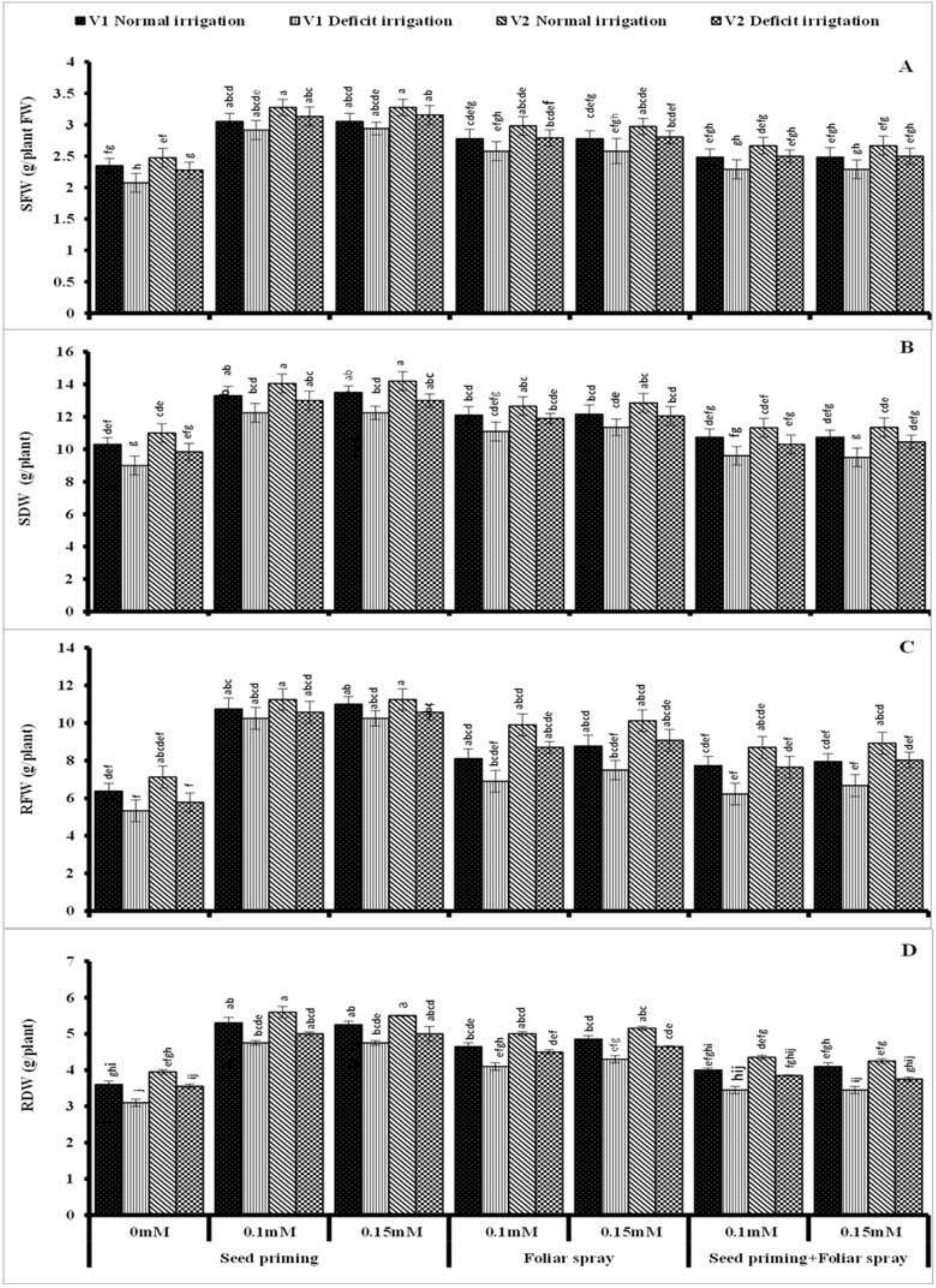

■ V1 Normal irrigation
▥ V1 Deficit irrigation
▧ V2 Normal irrigation
▩ V2 Deficit irrigtation
A
SFW (g/plant FW)
0
0.5
1
1.5
2
2.5
3
3.5
4
fg
h
ef
g
abcd
abcde
a
abc
abcd
abcde
a
ab
cdefg
efgh
abcde
bcdef
cdefg
efgh
abcde
bcdef
efgh
gh
defg
efgh
efgh
gh
efg
efgh
B
SDW (g/plant)
0
2
4
6
8
10
12
14
16
def
g
cde
efg
ab
bcd
a
abc
ab
bcd
a
abc
bcd
cdefg
abc
bcde
bcd
cde
abc
bcd
defg
fg
cdef
efg
defg
g
cde
defg
C
RFW (g/plant)
0
2
4
6
8
10
12
14
def
f
abcdef
f
abc
abcd
a
abcd
ab
abcd
a
abc
abcd
bcdef
abcd
abcde
abcd
bcdef
abcd
abcde
cdef
ef
abcde
def
cdef
ef
abcd
def
D
RDW (g/plant)
0
1
2
3
4
5
6
7
ghi
j
efgh
ij
ab
bcde
a
abcd
ab
bcde
a
abcd
bcde
efgh
abcd
def
bcd
cfg
abc
cde
efghi
hij
defg
fghij
efgh
ij
efg
ghij
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

Figure 2

SL, RL, H_2O_2, MDA of differentially drought tolerant mungbean genotypes fertigated with different levels of ALA through different modes when grown under normal irrigation and deficit irrigation.

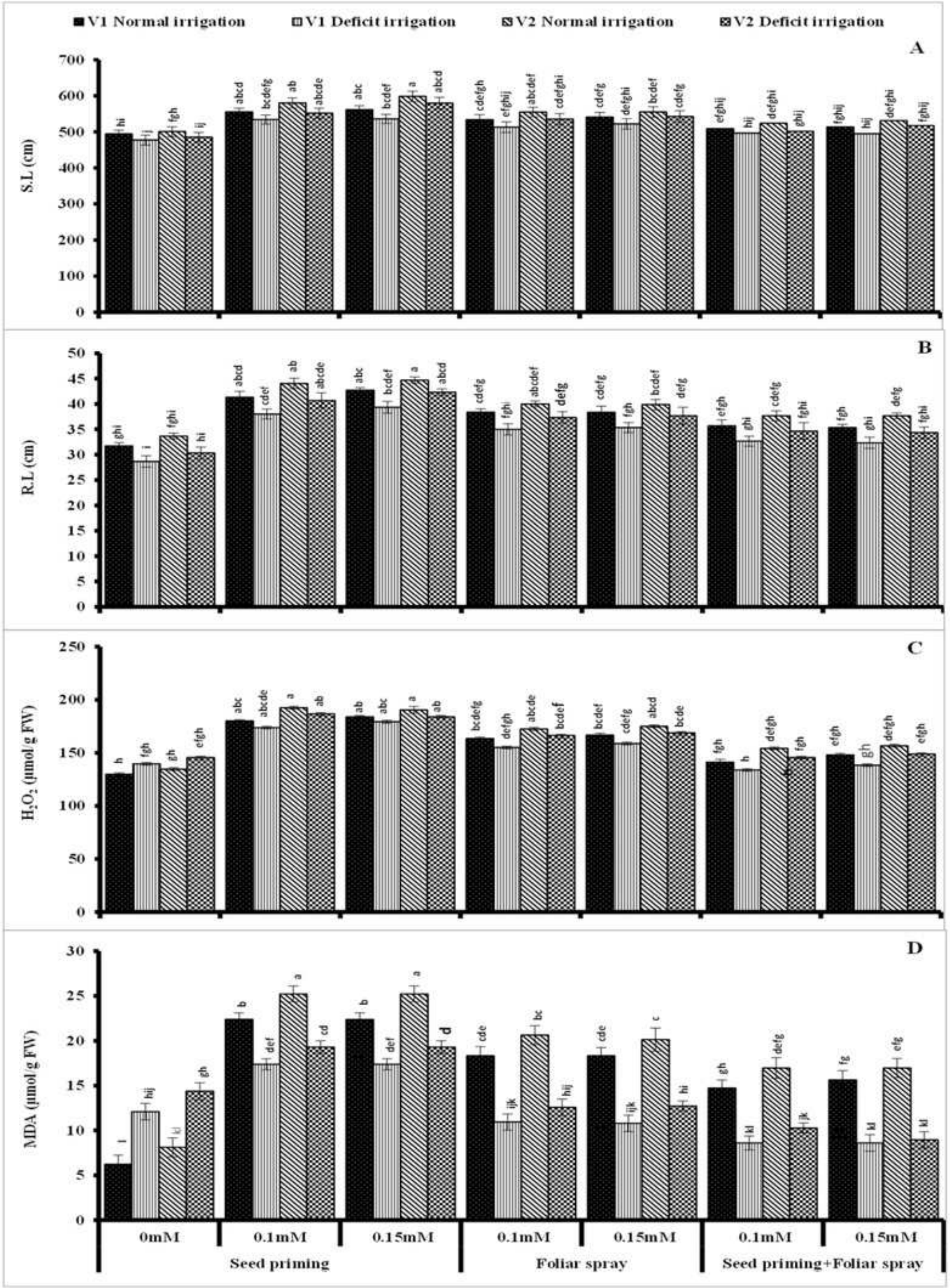
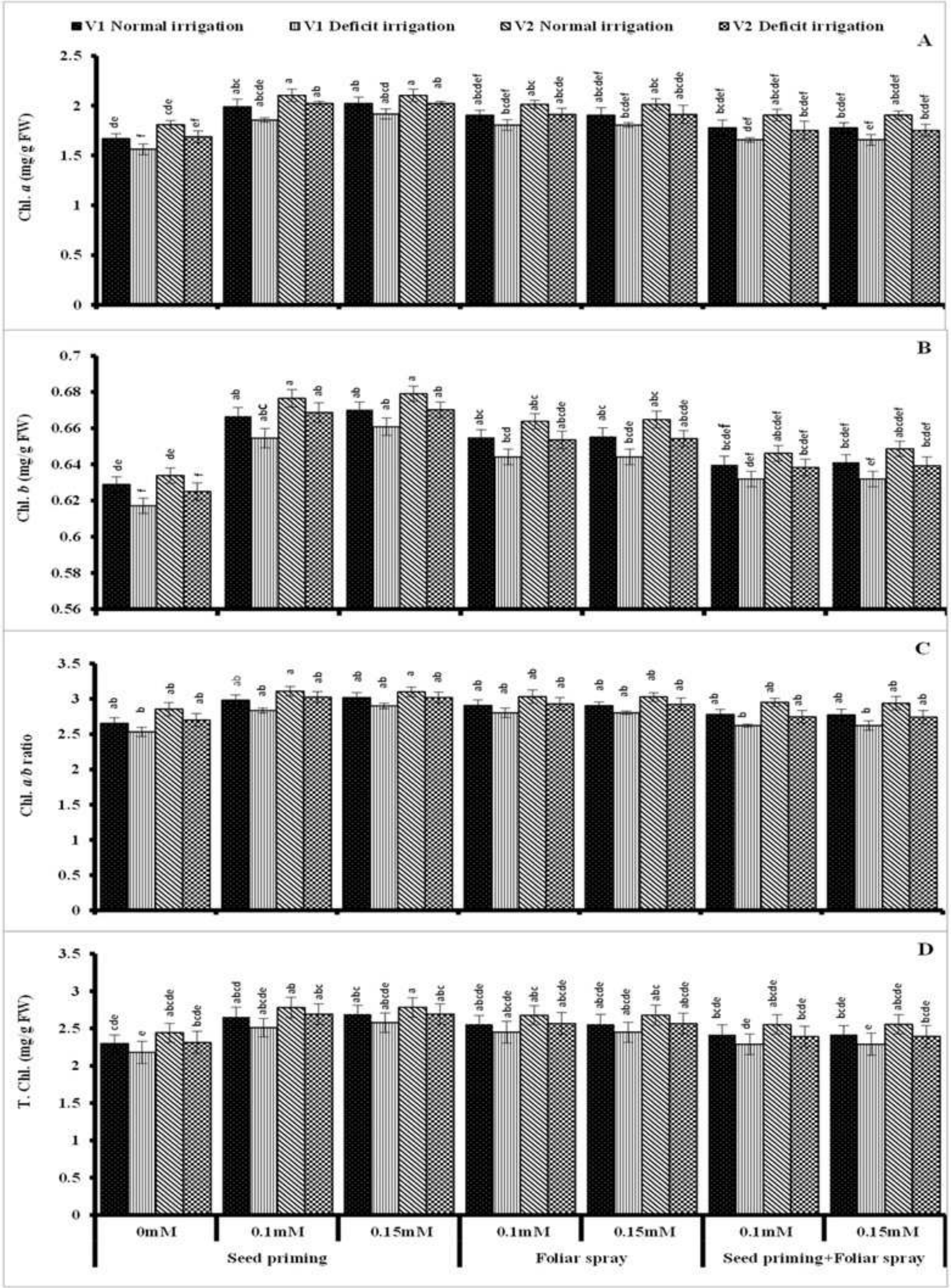

■V1 Normal irrigation
▥V1 Deficit irrigation
▧V2 Normal irrigation
▩V2 Deficit irrigation
A
S.L (cm)
0
100
200
300
400
500
600
700
hi
ij
fgh
ij
abcd
bcdefg
ab
abcde
abc
bcdef
a
abcd
cdefgh
efghij
abcdef
cdefghi
cdefg
defghi
bcdef
cdefg
efghij
hij
defghi
ghij
fghij
hij
defghi
fghij
B
R.L (cm)
0
5
10
15
20
25
30
35
40
45
50
ghi
i
fghi
hi
abcd
cdef
ab
abcde
abc
bcdef
a
abcd
cdefg
fghi
abcdef
defg
cdefg
fgh
bcdef
defg
efgh
ghi
cdefg
fghi
fgh
ghi
defg
fghi
C
H_2O_2 (µmol/g FW)
0
50
100
150
200
250
h
fgh
gh
efgh
abc
abcde
a
ab
ab
abc
a
ab
bcdefg
defgh
abcde
bcdef
bcdef
cdefg
abcd
bcde
fgh
h
defgh
fgh
efgh
gh
defgh
efgh
D
MDA (µmol/g FW)
0
5
10
15
20
25
30
l
hij
kl
gh
b
def
a
cd
b
def
a
d
cde
ijk
bc
hij
cde
ijk
c
hi
gh
kl
defg
jk
fg
kl
efg
kl
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

Figure 3

Chl. *a*, Chl. *b*, Chl. *a/b*, T. Chl of differentially drought tolerant mungbean genotypes fertigated with different levels of ALA through different modes when grown under normal irrigation and deficit irrigation


■ V1 Normal irrigation
▥ V1 Deficit irrigation
▧ V2 Normal irrigation
▩ V2 Deficit irrigation
A
Chl. a (mg/g FW)
2.5
2
1.5
1
0.5
0
de f cde ef
abc abcde a ab
ab abcd a ab
abcdef bcdef abc abcde
abcdef bcdef abc abcde
bcdef def abcdef bcdef
bcdef ef abcdef bcdef
B
Chl. b (mg/g FW)
0.7
0.68
0.66
0.64
0.62
0.6
0.58
0.56
de f de f
ab abC a ab
ab ab a ab
abc bcd abc abcde
abc bcde abc abcde
bcdef def abcdef bcdef
bcdef ef abcdef bcdef
C
Chl. a/b ratio
3.5
3
2.5
2
1.5
1
0.5
0
ab b ab ab
ab ab a ab
ab ab a ab
ab ab ab ab
ab ab ab ab
ab b ab ab
ab b ab ab
D
T. Chl. (mg/g FW)
3.5
3
2.5
2
1.5
1
0.5
0
cde e abcde bcde
abcd abcde ab abc
abc abcde a abc
abcde abcde abc abcde
abcde abcde abc abcde
bcde de abcde bcde
bcde e abcde bcde
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

.

Figure 4

Glycine betaine, AsA, proline, TPC of differentially drought tolerant mungbean genotypes fertigated with different levels of ALA through different modes when grown under normal irrigation and deficit irrigation.

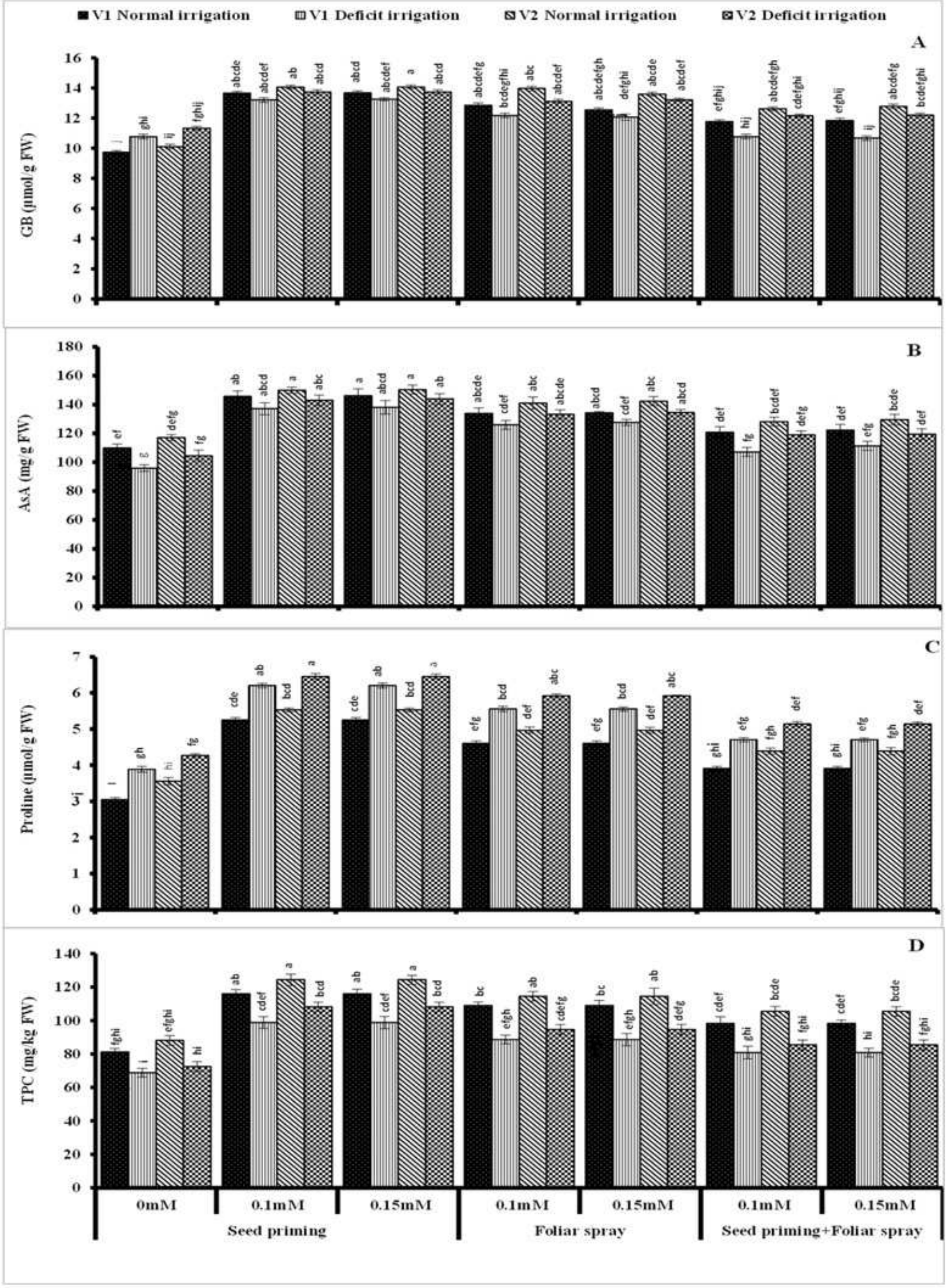

■ V1 Normal irrigation
▥ V1 Deficit irrigation
▧ V2 Normal irrigation
▩ V2 Deficit irrigation
A
GB (µmol/g FW)
0
2
4
6
8
10
12
14
16
j
ghi
ij
fghij
abcde
abcdef
ab
abcd
abcd
abcdef
a
abcd
abcdefg
bcdefghi
abc
abcdef
abcdefgh
defghi
abcde
abcdef
efghij
hij
abcdefgh
cdefghi
efghij
ij
abcdefg
bcdefghi
B
AsA (mg/g FW)
0
20
40
60
80
100
120
140
160
180
ef
g
defg
fg
ab
abcd
a
abc
a
abcd
a
ab
abcde
cdef
abc
abcde
abcd
cdef
abc
abcd
def
fg
bcdef
defg
def
efg
bcde
def
C
Proline (µmol/g FW)
0
1
2
3
4
5
6
7
i
gh
hi
fg
cde
ab
bcd
a
cde
ab
bcd
a
efg
bcd
def
abc
efg
bcd
def
abc
ghi
efg
fgh
def
ghi
efg
fgh
def
D
TPC (mg/kg FW)
0
20
40
60
80
100
120
140
fghi
i
efghi
hi
ab
cdef
a
bcd
ab
cdef
a
bcd
bc
efgh
ab
cdefg
bc
efgh
ab
defg
cdef
ghi
bcde
fghi
cdef
hi
bcde
fghi
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

Figure 5

TSP, TFC of differentially drought tolerant mungbean genotypes fertigated with different levels of ALA through different modes when grown under normal irrigation and deficit irrigation.

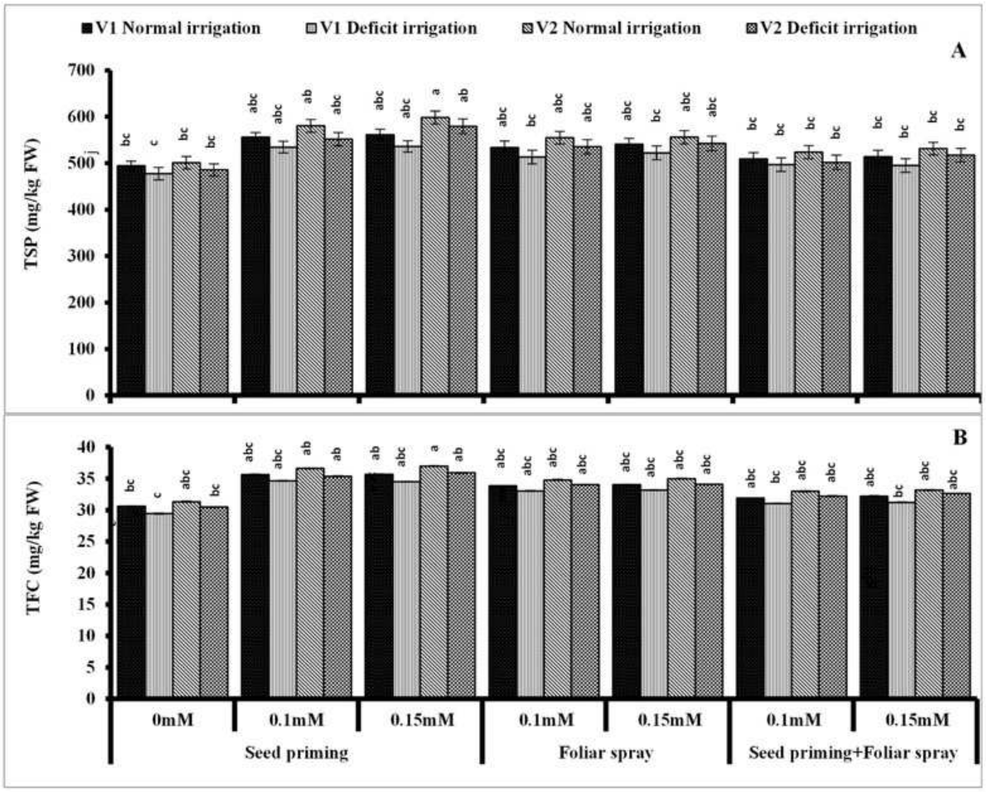

■V1 Normal irrigation
▥V1 Deficit irrigation
▧V2 Normal irrigation
▩V2 Deficit irrigation
A
TSP (mg/kg FW)
700
600
500
400
300
200
100
0
bc
c
bc
bc
abc
abc
ab
abc
abc
abc
a
ab
abc
bc
abc
abc
abc
bc
abc
abc
bc
bc
bc
bc
bc
bc
bc
bc
B
TFC (mg/kg FW)
40
35
30
25
20
15
10
5
0
bc
c
abc
bc
abc
abc
ab
ab
ab
abc
a
ab
abc
abc
abc
abc
abc
abc
abc
abc
abc
bc
abc
abc
abc
bc
abc
abc
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

Figure 6

Activities of CAT, SOD and POD of differentially drought tolerant mungbean genotypes fertigated with different levels of ALA through different modes when grown under normal irrigation and deficit irrigation.

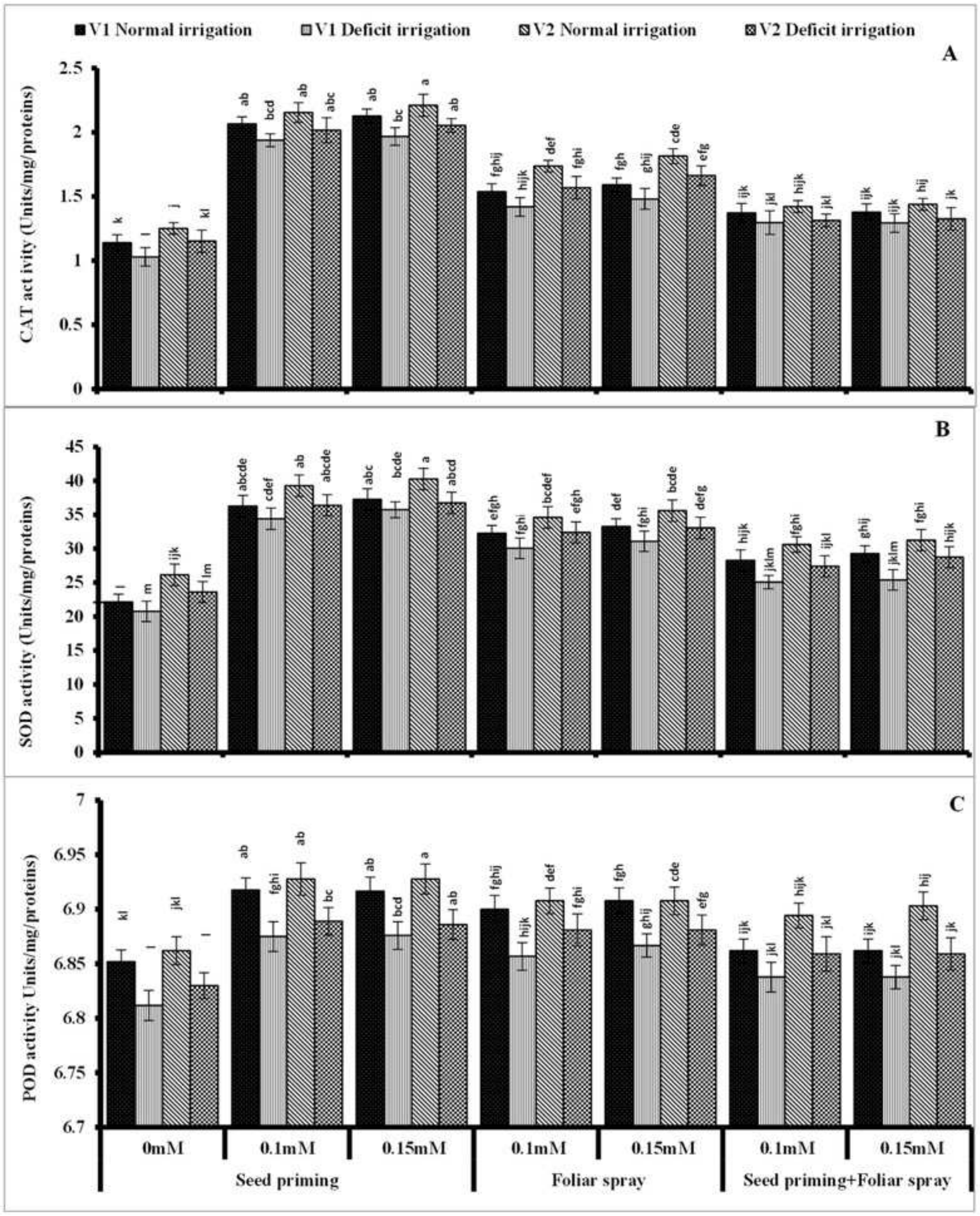

■ V1 Normal irrigation
▥ V1 Deficit irrigation
▧ V2 Normal irrigation
▩ V2 Deficit irrigation
A
CAT act ivity (Units/mg/proteins)
0
0.5
1
1.5
2
2.5
k
l
j
kl
ab
bcd
ab
abc
ab
bc
a
ab
fghij
hijk
def
fghi
fgh
ghij
cde
efg
ijk
jkl
hijk
jkl
ijk
ijjk
hij
jk
B
SOD activity (Units/mg/proteins)
0
5
10
15
20
25
30
35
40
45
l
m
ijk
lm
abcde
cdef
ab
abcde
abc
bcde
a
abcd
efgh
fghi
bcdef
efgh
def
fghi
bcde
defg
hijk
jklm
fghi
ijkl
ghij
jklm
fghi
hijk
C
POD activity Units/mg/proteins)
6.7
6.75
6.8
6.85
6.9
6.95
7
kl
l
jkl
l
ab
fghi
ab
bc
ab
bcd
a
ab
fghij
hijk
def
fghi
fgh
ghij
cde
efg
ijk
jkl
hijk
jkl
ijk
jkl
hij
jk
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

Figure 7

Number of seeds per pod, number of pods per plant, 100GW, TGY of differentially drought tolerant mungbean genotypes fertigated with different levels of ALA through different modes when grown under normal irrigation and deficit irrigation.

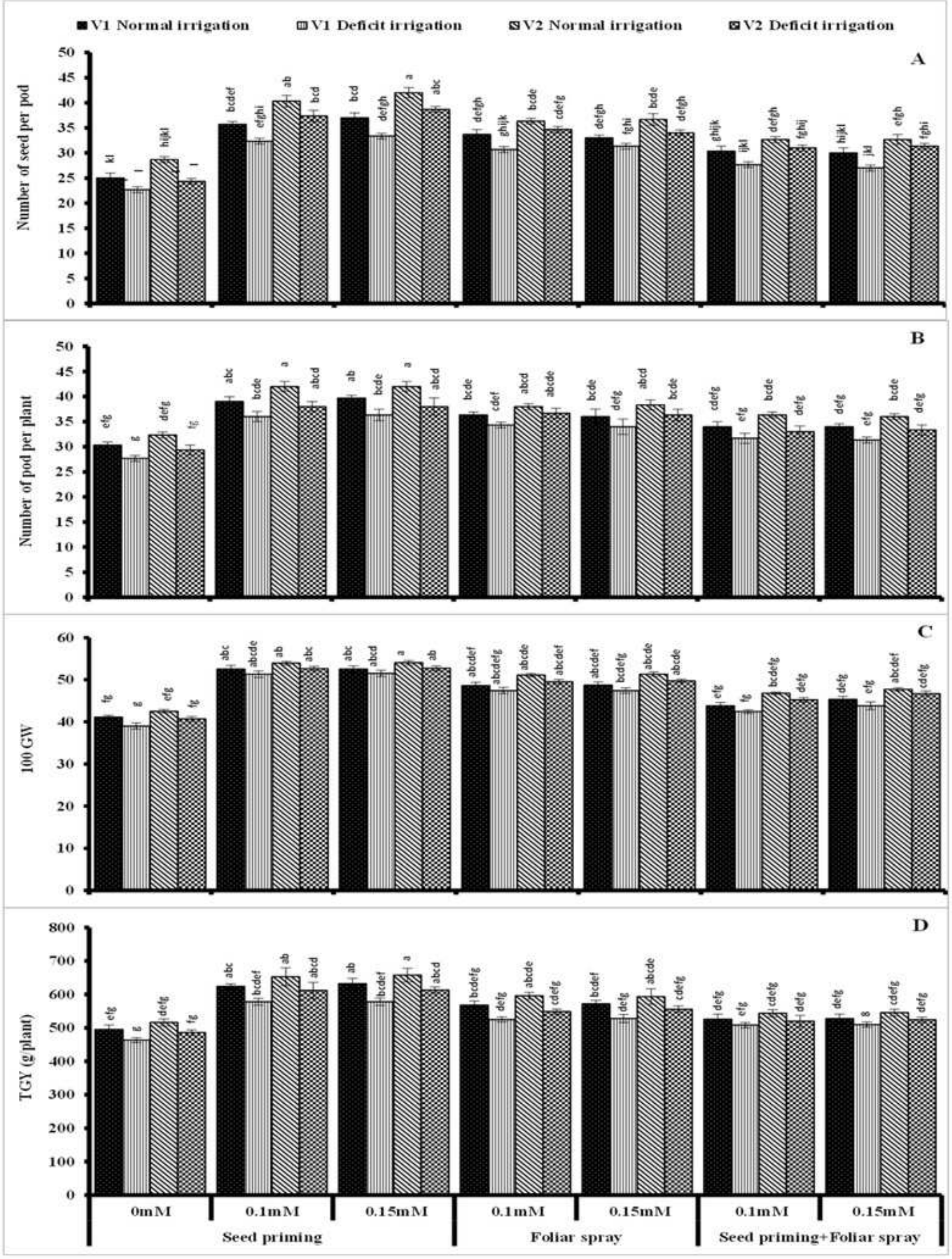

■V1 Normal irrigation
▥V1 Deficit irrigation
▧V2 Normal irrigation
▩V2 Deficit irrigation
A
Number of seed per pod
0
5
10
15
20
25
30
35
40
45
50
kl
l
hijkl
l
bcdef
efghi
ab
bcd
bcd
defgh
a
abc
defgh
ghijk
bcde
cdefg
defgh
fghi
bcde
defgh
ghijk
ijkl
defgh
fghij
hijkl
jkl
efgh
fghi
B
Number of pod per plant
0
5
10
15
20
25
30
35
40
45
50
efg
g
defg
fg
abc
bcde
a
abcd
ab
bcde
a
abcd
bcde
cdef
abcd
abcde
bcde
defg
abcd
bcde
cdefg
efg
bcde
defg
defg
efg
bcde
defg
C
100 GW
0
10
20
30
40
50
60
fg
g
efg
fg
abc
abcde
ab
abc
abc
abcd
a
ab
abcdef
abcdefg
abcde
abcdef
abcdef
bcdefg
abcde
abcde
efg
fg
bcdefg
defg
defg
efg
abcdef
cdefg
D
TGY (g/plant)
0
100
200
300
400
500
600
700
800
efg
g
defg
fg
abc
bcdef
ab
abcd
ab
bcdef
a
abcd
bcdefg
defg
abcde
cdefg
bcdef
defg
abcde
cdefg
defg
efg
cdefg
defg
defg
g
cdefg
defg
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

Figure 8

. Heatmap histogram correlation between different studied attributes of mung bean line (V16003) fertigated with different levels of lipoic acid

priming [(Control 0mM (1), 0.1mM (2), 0.15mM (3), stress 0mM (4), 0.1mM (5), 0.15mM (6)], foliar [Control 0.1mM (7), 0,15mM (8), stress (0mM (9), 0.1mM (10), 0.15mM (11)] and priming+ foliar [Control 0.1mM (12), 0.15mM (13), stress 0mM (14), 0.1mM (15), 0.15mM (16)] when grown under normal irrigation and deficit irrigation

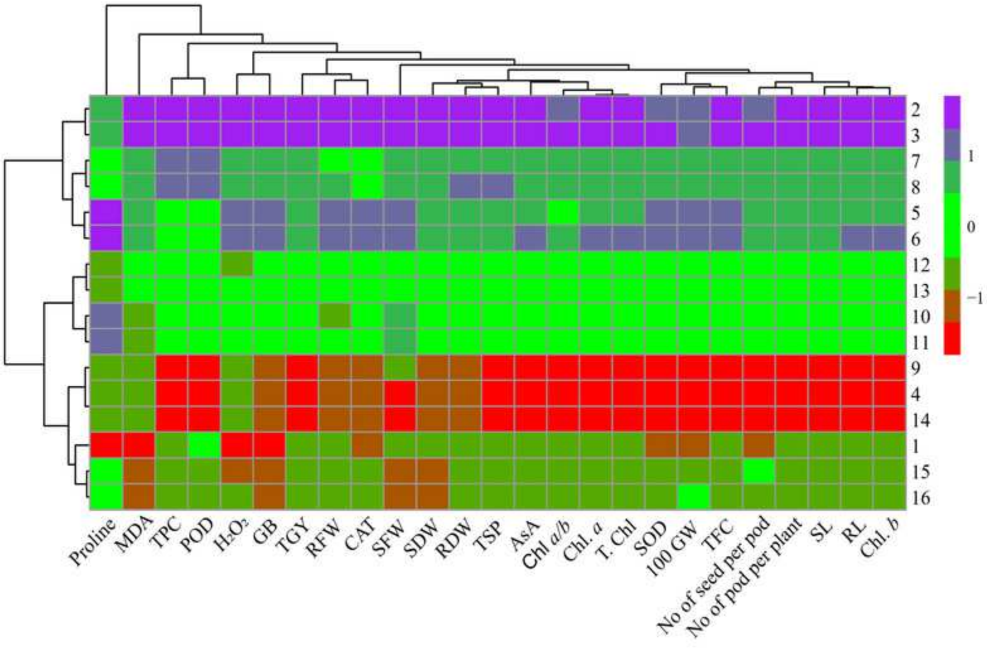
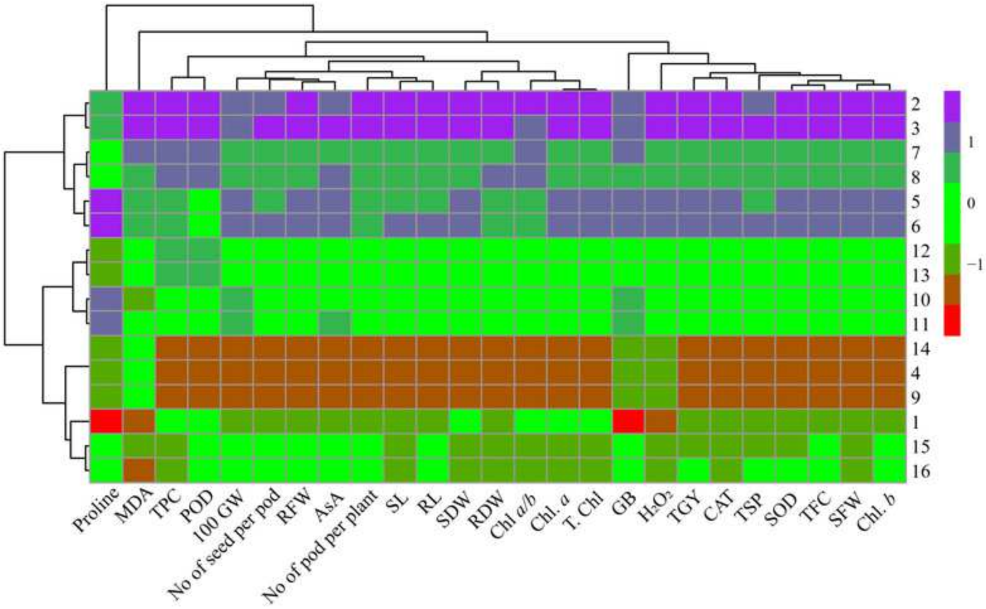

2
3
7
8
5
6
12
13
10
11
9
4
14
1
15
16
1
0
−1
Proline
MDA
TPC
POD
H₂O₂
GB
TGY
RFW
CAT
SFW
SDW
RDW
TSP
AsA
Chl a/b
Chl. a
T. Chl
SOD
100 GW
TFC
No of seed per pod
No of pod per plant
SL
RL
Chl. b

Figure 9

Heatmap histogram correlation between different studied attributes of mung bean lines (V16004) fertigated with different levels of lipoic acid

priming [(Control 0mM (1), 0.1mM (2), 0.15mM (3), stress 0mM (4), 0.1mM (5), 0.15mM (6)], foliar [Control 0.1mM (7), 0.15mM (8), stress (0mM (9), 0.1mM (10), 0.15mM (11)] and priming+ foliar [Control 0.1mM (12), 0.15mM (13), stress 0mM (14), 0.1mM (15), 0.15mM (16)] when grown under normal irrigation and deficit irrigation


2
3
7
8
5
6
12
13
10
11
14
4
9
1
15
16
1
0
−1
Proline
MDA
TPC
POD
100 GW
No of seed per pod
RFW
AsA
No of pod per plant
SL
RL
SDW
RDW
Chl *a/b*
Chl. *a*
T. Chl
GB
H_2O_2
TGY
CAT
TSP
SOD
TFC
SFW
Chl. *b*