Alpha lipoic acid mitigates adverse impacts of drought stress on growth and yield of mungbean: photosynthetic pigments, and antioxidative defense mechanism (#92451)

First revision

Guidance from your Editor

Please submit by **28 Jan 2024** for the benefit of the authors .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files
Download and review all files from the materials page.

1 Tracked changes manuscript(s)
1 Rebuttal letter(s)
9 Figure file(s)
1 Raw data file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Alpha lipoic acid mitigates adverse impacts of drought stress on growth and yield of mungbean: photosynthetic pigments, and antioxidative defense mechanism

Naima Hafeez Mian [1], **Muhammad Azeem** [Corresp., 1], **Qasim Ali** [1], **Saqib Mahmood** [1], **Muhammad Sohail Akram** [1]

[1] Government College University, Faisalabad, Faisalabad, Pakistan

Corresponding Author: Muhammad Azeem
Email address: mazeem@gcuf.edu.pk

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** The present study aimed to explore the possible role of alpha lipoic acid (ALA) to induce water stress tolerance in mungbean lines when applied exogenously through different modes. **Methods** The experiment was conducted in a field with split split plot arrangement having three replicates for each treatment. Two regimes of irrigation including normal irrigation and reduced irrigation were applied. The plants allocated to reduced irrigation were watered only at the reproductive stage. Three levels of ALA (0 mM, 0.1 mM, 0.15 mM) were applied through different modes (seed priming, foliar or priming+foliar). **Key results** ALA treatment through different modes manifested higher growth both under reduced irrigation (water stress) and normal irrigation. 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, maintenance of plant water relations, levels of non-enzymatic antioxidants, improved activities of enzymatic antioxidants, and decreased lipid peroxidation and H_2O_2 levels. The maximum increase in SFW (29 and 28%) and SDW (27 and 24%) and in 100 GW (24 and 23%) and TGY (20 and 21%) in water stressed mungbean plants of line 16003 and 16004 respectively was recorded due to ALA seed priming than other modes of applications **Conclusions** Conclusively, 0.1, 0.15 mM 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 of the study will be helpful for the agriculturalists working in arid and semi-arid regions to obtain the better yield of mungbean that will be helpful to fulfill the food demand in those areas up to some extent .

Alpha lipoic acid mitigates adverse impacts of drought stress on growth and yield of mungbean: photosynthetic pigments, and antioxidative defense mechanism

Naima Hafeez Mian[1], Muhammad Azeem[1*], Qasim Ali[1], Saqib Mahmood[1], Muhammad Sohail Akram[1]

[1]Department of Botany, Government College University, Faisalabad, Pakistan

*Corresponding author email: mazeem@gcuf.edu.pk

ABSTRACT

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

The present study aimed to explore the possible role of alpha lipoic acid (ALA) to induce water stress tolerance in mungbean lines when applied exogenously through different modes.

Methods

The experiment was conducted in a field with split split plot arrangement having three replicates for each treatment. Two regimes of irrigation including normal irrigation and reduced irrigation were applied. The plants allocated to reduced irrigation were watered only at the reproductive stage. Three levels of ALA (0 mM, 0.1 mM, 0.15 mM) were applied through different modes (seed priming, foliar or priming+foliar).

Key results

ALA treatment through different modes manifested higher growth both under reduced irrigation (water stress) and normal irrigation. 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, maintenance of plant water relations, levels of non-enzymatic antioxidants, improved activities of enzymatic antioxidants, and decreased lipid peroxidation and H_2O_2 levels. The maximum increase in SFW (29 and 28%) and SDW (27 and 24%) and in 100 GW (24 and 23%) and TGY (20 and 21%) in water stressed mungbean plants of line 16003 and 16004 respectively was recorded due to ALA seed priming than other modes of applications

Conclusions

Conclusively, 0.1, 0.15 mM 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 of the study will be helpful for the agriculturalists working in arid and semi-arid regions to obtain the better yield of mungbean that will be helpful to fulfill the food demand in those areas up to some extent.

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

INTRODUCTION

Vigna radiata L. (Wilczek) (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* L. (Wilczek) 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., 2014*). Water stress is one of the principal environmental stresses that hinders plant growth, development, and crop productivity; especially in arid and semi-arid environments (*dos Santos et al., 2022*).

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 metabolites (*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 (*Hasanuzzaman et al., 2021*).

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 (EC 1.11.1.6), ascorbate peroxidase (APX) (EC 1.11.1.11), guaiacol peroxidase (GPX) (EC 1.11.1.7) and SOD (EC 1.15.1.1) (*Rajput et al., 2021*).

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 (*Youssef et al., 2021; Akhter et al., 2023; Ramadan et al., 2022*). Amongst these, ALA is also considered as one of the novel substances among others but few recent reports are available. Mostly these studies are conducted in pots rather than in field conditions focusing the salt, osmotic, and heavy metal stresses (*Terzi et al., 2018; Youssef et al., 2021; Mian et al., 2021; Rajput et al., 2021; Ramadan et al., 2022*), while the present study was conducted under field water deficit conditions. Moreover, earlier studies were focusing on earlier vegetative stages, either the attributes studied

or in case of ALA exogenous application. Whereas in present study the ALA was applied as seed priming or as foliar spray at the vegetative stage

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*). It is 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; Alomran et al., 2023*). 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 (*Rajput et al., 2021; 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 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 on seed yield of mungbean lines. The objectives of the study were to find out the ameliorative impacts of deficit irrigation on 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 were also the selection of most effective level of ALA either applied as seed priming, foliar spray or combination of both for better seed yield of mungbean in field conditions under deficit irrigation that is still not found yet.

MATERIALS AND METHODS

Experimental conditions

Present experiment was conducted to assess the response of two high yielding mung bean lines (16003 and 16004) to exogenously supplied ALA through different modes when grown under field water deficit conditions. Line 16003 is a drought sensitive one while the line 16004 is a moderately drought tolerant (*Anonymous, 2019*). 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 regimes of irrigation i.e., normal irrigation (3 irrigation during growth period) and reduced irrigation (water stress) (only once). 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 regime (normal irrigation and reduced irrigation). Each main plot was divided to two subplots each specific for mungbean line. 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 6 m length with a furrow-to-furrow distance of 60 cm. A set of three furrows was specified for each specific level of ALA (0 mM, 0.1 mM, 0.15 mM of ALA). These doses of ALA as exogenous use were selected based on the available studies regarding its exogenous use (*Elrys et al., 2020*; *Yadav et al., 2022*). For seed priming treatment the seeds of both mungbean lines were primed separately with each specific level of ALA solution for 6 h 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 15 cm 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 30 cm plant to plant distance. During ploughing and land preparation the soil was supplied with adequate amount of fertilizer [N (160 Kg/ha), P (80 Kg/ha) and K (50 Kg/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 milliliters of solution of ALA of each treatment were 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 were collected after two weeks of application 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) 972 $\mu molm^{-2}s^{-1}$ 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 h.

Determination of leaf photosynthetic pigments

The contents of Leaf Chl. *a* and Chl. *b* were estimated by using (*Arnon, 1949*) approach. An extract of 0.5 g of fresh leaf was made in 10 ml 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 163 Chl. *a* (measured in mg g-1 FW) = [12.7 (OD 663) -2.69 (OD 645)] x V/1000 x W 164 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 & Allen, 1965)* was used.

Carotenoids (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 0.5 g dry leaf material using 10 ml of distilled water and kept it over-night at 4 ℃. Centrifuged the samples at 10,000 × *g* for 10 minutes. Then the 1 ml of the extract and 1 ml of 2 N 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 0.25 g thawed in 10 ml of 3% sulfosalicylic acid solution and filtered the mixture. Then, added 2 ml of filtrate into the test tube and 2 ml of acid ninhydrin along with 2 ml 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 (30 ml) and 6 *M* ortho--phosphoric acid (20 ml). Then the resultant mixture was incubated at 100 ℃ for an hour. Cool down the solution and 2 ml toluene were 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 (0.5 g) was homogenized in 50 mM 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 (1 ml) 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 (0.25 g) was ground in 10 ml of 6% TCA solution. After centrifugation the supernatant (4 ml) was mixed with 2 ml of 2% dinitrophenyl hydrazine in prepared in 9N H_2SO_4. One drop of 10% thiourea was added to the solution. The thiourea solution was prepared by mixing the 2 g 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 5 ml 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 2 ml distilled water along with Folin-Ciocalteu's phenol reagent (1 ml) and shake gently. Then to the above mixture 5 ml of 20% Na_2CO_3 was added and the final volume was made up to 10 ml 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 ml of 80% aqueous methanol. The filtrate was then obtained by filtering. Add 0.3 ml of 5% $NaNO_2$ 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% $AlCl_3$ 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 (0.1 ml) was reacted with 2 ml 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 (0.25 g) was ground in 1% TCA (3 ml) at room temperature. At 5000 × *g* centrifuged the extract for 15 minutes. Then 1 ml of supernatant was mixed with 4 ml of 0.5% TBA prepared in 20% TCA solution. Incubate the solution at 95 °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 50 minutes 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 (100 GW) 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. By using R-studio (Version 4.2.2) principal component analysis among studied attributes and heat map to detect the correlation among studied parameters and treatments with parameters were constructed.

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 & 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 0.1 mM, 0.15 mM 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 line (16004) under both water stress and non-stress conditions. Foliar application of 0.15 mM 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 mM, 0.15 mM) 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. Line 16003 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 mM, 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 mM, 0.15 mM) 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 mM, 0.15 mM) 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 AsA contents of both mungbean lines 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 mM, 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 treatment was found more effective approach in increasing the leaf AsA contents in both mungbean lines when 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 (0.1 mM, 0.15 mM) 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 (0.1 mM, 0.15 mM) 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 0.1 mM, 0.15 mM 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 (0.1 mM, 0.15 mM) 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 these studied antioxidant enzymes showed significant increase in plants raised from seeds primed with 0.1 mM, 0.15 mM 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, 100 GW 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 (0.1 mM, 0.15 mM) 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 lines to water deficit stress but also corresponds well the responses of line 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 line 16003 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 line 16004 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 line 16004 in comparisons to line 16003. Heatmap histogram based on intensities of colors (blue color) shows that a strong positive correlation can be observed of catagories 2 (0.1 mM), 3 (0.15 mM), 5 (0.1 mM) and 6 (0.15 mM) 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 line 16004. Catagories 7 and 8 corresponding to foliar treatments of 0.1 mM, 0.15 mM ALA also showed positive influence but were less than the pre-sowing seed treatment. Foliar spray of ALA 0.1 mM, 0.15 mM under stress and the combined treatments were not found so positively effective in both mungbean lines 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 water for irrigation. Under deficiency irrigation plant undergo numerous metabolic modifications for their survival in present investigation, significant decrease in growth and yield was recorded in 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 on growth of canola, wheat, quinoa, and moringa plants that was associated with increased lipid peroxidation due to overly produced ROS, reduced cell enlargement due to disturbed water relation (*Shahid et al., 2022*). Water stress induced negative impact on plant water relations causes osmotic stress that

directly effects the, imbibition process necessary for cell expansion leading to cell division responsible for continuity of growth (*Shehzad et al., 2022*; *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 growth and yield (*Seleiman et al., 2021*) similar is in present investigation. However, this reductions in growth and yield attributes 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 due to involvement in different physiological and biochemical activities. Earlier studies revealed the promotive role of ALA on growth attributes of plants grown under stress (*Youssef et al., 2021*) who reported that ALA act as a growth regulator and reported as a protector against abiotic stress (*Ramadan et al., 2022*). Moreover, it is reported earlier that exogenous application of ALA promoted root system in canola seedlings grown under stress (*Javeed et al., 2021*) that was found helpful in promoting the plant shoot system and growth attributes associated with increased uptake of nutrients and water leading to modulation in physiological process such as (*Ramadan et al., 2022*) photosynthesis and increased antioxidant activities (*Youssef et al., 2021*). Depicts earlier findings in present experiment it was found that ALA acts as a potential modulator of plant growth under stress in a mode of application-dependent manner, where seed priming mode of application found superior one as compared to the foliar or foliar plus priming treatment, for better growth and yield.

Moreover, ALA improved yield has already been reported earlier was (*Xiao et al., 2018*; *Elkelish et al., 2021*) alpha lipoic acid induced application as potential antioxidant reported is to accelerate the growth of plants associated with cell enlargement, cell division due to maintenance in membrane integrity and reduced ion linkage (*Muscolo et al., 2014*; *Hassan et al., 2021*). In plants photosynthetic pigments such as chlorophylls are indispensable to maintain optimum photosynthetic efficiency in plant by harvesting sun light (*Tabassum et al., 2016*). Under water stress conditions, leaf chlorophylls contents disturbs seriously but plant species specific (*Zargar et al., 2017*; *Shin et al., 2021*) and water stress intensity specific (*Zargar, 2017; Wang et al., 2016; Hussain et al., 2018; Tahir et al., 2019; Ma et al., 2022*) as well as in mungbean cultivars. (*Uddin et al., 2021*) that corresponds well to present findings. Its results in stomatal closure leading to one relation in gas exchange attributes in associated with 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 responsible for the biosynthesis of chlorophyll molecules due to decreased *de novo* synthesis of said pigments and further the destruction of the pigment protein complexes (*Paim et al., 2020*) chloroplast lipids also contribute in declining the Chl. *a,* Chl. *b* and carotenoids contents (*Liang et al., 2020*; *Uddin et al., 2021*).

In the present study, exposure of mungbean plants to water deficit conditions noticeably altered the leaf chlorophyll contents. It was found 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 to the disturbed activity of chlorophyllase (*Ali et al., 2018*) as well as oxidative damages (*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 that shows the antistress roles of ALA.

Similar ameliorations in stress induced damages to pigments by exogenously-applied ALA has previously been reported, where ALA induced improvement in Chl. *a*, Chl. *b* and T. Chl. contents under stressful condition associated with reduced lipid peroxidation by stimulating the antioxidant systems in wheat and maize (*Sezgin et al., 2019*; *Elkelish et al., 2021; Ramadan et al., 2022*). This indicates the species-specific role of ALA. Moreover, (*Yousaf et al.*, *2021*) reported that ALA application maintains the ultra-structure of chloroplasts for preserving the chlorophyll that proves the promoting role of ALA in photosynthesis under water stress (*Youssef et al., 2021*).

Due to sulfur-containing biomolecules ALA considered as very effective antioxidants to protect the plants when grown under abiotic stresses (*Gorcek & Erdal, 2015*). It was found that exogenous application of ALA stimulates not only the PS- 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 with a concurrent down-regulation in the chlorophyllase gene (Chlase) and increase N uptake, essential for the biosynthesis of chlorophyll (*Sezgin et al., 2019; 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.

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 variety of organic molecules that improve plant water relations by performing role as osmo-regulators and to avoid disintegration of protein working as crops (*Majumdar et al., 2016; Alnusairi et al., 2021*; *Ibrahim et al., 2021*; *Nahhas et al., 2021; Shao et al., 2021; Irshad et al., 2022*). Accumulation of proline maintains the integrity of membrane decreasing lipid oxidation through scavenging free radicals (*Shinde et al., 2016*) and act as a signaling compound by regulating the function of mitochondria and controls the proliferation of cell by activating particular antistress genes (*Meena et al., 2019*). In present study imposition of water stress caused a marked increase in leaf proline and GB accumulation in both mungbean lines showing their osmoregulation potential.

In current study exogenously-applied ALA further increased the accumulation of proline and GB in mungbean plants of both lines under water stress, that are in agreement with former reports on different crop plants (*Mohammad-khani & Heidari, 2008; Terzi et al., 2018*; *Sezgin et al., 2019*; *Elkelish et al., 2021*; *Youssef et al., 2021*) where they reported the role of ALA in plant water relation through osmotic adjustment. Opposite to our findings a reduction in leaf proline content were recorded in ALA treated stressed wheat plants (*Ramadan et al*., *2022)*.

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; Shehzad et al., 2022*). In the present study water stress significantly increased leaf H_2O_2 content but the exogenously-applied ALA in any mode significantly decreased its level that is in agreement to previous studies (*Youssef et al., 2021*; *Ramadan et al., 2022*) where ALA application reduced the lipid peroxidation by playing anti-stress role. However, the interaction between increased ROS levels and ALA remains unknown under water stress, as limited literature is available regarding this treatment. To scavenge the overly produced ROS, plants have developed antioxidant defense mechanism compounds responsive for the reduction of lipid peroxidation but strongly cultivar /species

specific and is measured in terms of MDA content (*Ali & Ashraf, 2011; Ali et al., 2016; Shehzad et al., 2022*). In present study MDA content increased significantly in both mungbean lines under water stress but ALA application lowered the MDA levels as reported in stressed sorghum (*Youssef et al., 2021*) and wheat (*Ramadan et al., 2022*). Our results revealed that water stress induced the substantial damage to cell membrane as depicted by increased H_2O_2 levels in both mungbean. However, ALA-induced decline in MDA and H_2O_2 is an indicative of better antioxidant system in the treated plants as reported of in wheat and yeast (*Gorcek & Erdal, 2015*). 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*). ALA acts in the recycling of other oxidized radical scavengers such as AsA and GSH (*Navari-Izzo et al., 2002*). Thus, considered as a potential candidate of antioxidants, having the ability to mitigate the oxidative damages induced by abiotic stresses (*Terzi et al., 2018*). This positive role of ALA in improvement of antioxidative defense mechanism is associated with the improved osmotic adjustment, water relation less damage to photosynthetic pigments as a result better growth under water stress.

Under stresses AsA plays crucial roles in plants that are plant species specific (*Naz et al., 2022; Alizadeh et al., 2023*). In present study, water stress significantly decreased the leaf AsA contents however, exogenously-applied ALA in ant mode improved its content in both lines of mungbean best being more in case of seed priming. 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 their accumulation along with other secondary metabolites mitigates the oxidative damages (*Wang et al., 2016*; *Ahmad et al., 2019; Li et al., 2019; Yadav et al., 2021*). In present study water stress caused significant reduction TPC and TFC in both mungbean lines that are similar as reported earlier (*Krol et al.,2014; Ali et al., 2018; Kumar et al., 2023*) where significant reductions in TPC and TFC where the phenolic compounds are being involved in plant tolerance to stresses (*Ferreyra et al., 2012; Saeed et al., 2023*). 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 polyphenolics content is positively associated with their better growth, better photosynthesis pigmentation and reduced lipid peroxidation that shows the stress tolerance role of exogenously applied ALA in both mungbean lines 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 in both mungbean lines but reports depict that up or down regulation activities of antioxidant enzymes or plant genotypes specific and stress type specific (*Wang et al., 2012; Rai et al., 2021; Youssef et al., 2021; Bashir et al., 2023; Urmi et al., 2023*). In present study 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*) and in water stressed *Triticum aestivum* plants (*Elkelish et al., 2021*) that was associated with their stress tolerance in term of better growth due to better oxidative stress performance. From the present findings as well as the previous studies it is confined that ALA applied improved antioxidative mechanism by upregulation enzyme activities is specific to type of stress, ALA level and plant species (*Huang et al., 2019*; *Elkelish et al., 2021; Hassan et al., 2021; Youssef et al., 2021*).

It is further advocated that ALA contributes to instigate cellular redox management and ROS scavenging activities (*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 line by exogenous use of ALA through different modes in term of better growth and yield is associated with better maintenance of plant water relations through osmotic adjustment, improvement in antioxidative defense mechanism with reduced lipid peroxidation and reduced membrane leakage, maintenance of better photosynthetic pigments in relation with reduced lipid peroxidation and better antioxidative defense mechanism. 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. Thus, comprehensive molecular level studies in relation with biochemical mechanisms are needed to uncover role of ALA in plants grown under water stress conditions.

CONCLUSIONS

Data shows that against water stress, ALA has many protective aspects to enhance plant growth and yield through improvement in pigmentation, enzymatic and non-enzymatic antioxidative mechanisms with reduction in oxidative damage and improved water relation. Among different modes of ALA treatment growth and yield of water stressed mungbean plants was superior ones the grown from seeds treated with ALA than foliar and combined treatment. 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 be helpful for the farmers to get better mungbean production under deficit irrigation or in rainfed 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

ACKNOWLEDGEMENTS

The authors highly acknowledge the Experimental Botany Lab and Plant physiology Lab, Government College University Faisalabad, for providing the facilities and instruments for conducting the analysis.

Funding

This research did not receive any specific funding.

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 study 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

Anonymous. 2019. Annual research programme, Kharif 2019, Pulses Research Institute, Faisalabad. https://aari.punjab.gov.pk/system/files/APRW%20Kharif%202019%20.pdf

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

Akhter N, Noreen A, Saifullah S, Noman A, Shahnaz MM, Letuma PM, Kausar A, Siddique M, Hashem M, Alamri S, Al-zoubi MO, Saleem M, Khalid N, Aqeel, M. 2023. Salt ions mediated changes in biochemical and anatomical characteristics of *Brassica napus* can be countered with moringa leaf extract. *South African Journal of Botany* ***156*****:** 352-364. https://doi.org/10.1016/j.sajb.2023.03.040

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

Alomran MM, Noman A, Aqeel M, Khalid N, Maqsood MF, Akhter N, Arshad M, Alqahtani FM, Alzuaibr FM, Hashem M, Habeeb T, Al-Zoubi OM, Alotaibi MO. 2023. Relative biochemical and physiological docking of cucumber varieties for supporting innate immunity against *Podosphaera xanthii*. *Microbial Pathogenesis* ***184*****:** 106359. https://doi.org/10.1016/j.micpath.2023.106359

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. 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

Bashir R, Ramzan HN, Mahmood S, Awais M, Hassan S, Aqeel M, Noman, A. 2023. Lipoic acid positively regulates tomato growth and yield by improving organic osmolytes and antioxidant defense system under saline conditions. *Journal of Soil Science and Plant Nutrition* ***23*****(3):** 4691-4703. https://doi.org/10.1007/s42729-023-01385-2

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_2O_2. *Journal of Biochemistry and Molecular Biology Reports* **35 (6)**: 547-552. doi: 10.5483/bmbrep.2002.35.6.547

589 Chance B, Maehly AC. 1955. Assay of catalases and peroxidases. *Methods in Enzymology* **2**: 764-775.
590 https://doi.org/10.1016/S0076-6879(55)02300-8
591 Chen LG, Gottschalck J, Hartman A, Miskus D, Tinker R, Artusa A. 2019. Flash drought characteristics based on
592 US drought monitor. *Journal of Atmosphere* **10(9)**: 498. https://doi.org/10.3390/atmos10090498
593 dos Santos TB, Ribas AF, de Souza SGH, Budzinski IGF, Domingues DS. 2022. Physiological responses to
594 drought, salinity, and heat stress in plants: a review. *Stresses* **2(1)**: 113-135.
595 https://doi.org/10.3390/stresses2010009
596 Ebert AW. 2014. Potential of underutilized traditional vegetables and legume crops to contribute to food and
597 nutritional security, income and more sustainable production systems. *Sustainability* **6(1)**: 319-335.
598 https://doi.org/10.3390/su6010319
599 Elkelish A, El-Mogy MM, Niedbała G, Piekutowska M, Atia MA, Hamada MM, Ibrahim MF. 2021. Roles of
600 exogenous α-lipoic acid and cysteine in mitigation of drought stress and restoration of grain quality in
601 wheat. *Plants* **10(11)**: 2318. https://doi.org/10.3390/plants10112318
602 Elrys AS, Abdo AIE, Abdel-Hamed EMW, Desoky EM. 2020. Integrative application of licorice root extract or
603 lipoic acid with fulvic acid improves wheat production and defenses under salt stress conditions. *Ecotoxicology*
604 *and Environmental Safety* **190**: 110144, https://doi.org/10.1016/j.ecoenv.2019.110144.
605 Favero VO, Carvalho RH, Motta VM, Leite ABC, Coelho MRR, Xavier GR, Urquiaga S. 2021. *Bradyrhizobium* as
606 the only rhizobial inhabitant of mung bean (*Vigna radiata*) nodules in tropical soils: a strategy based on
607 microbiome for improving biological nitrogen fixation using bio-products. *Frontiers of Plant Science* **11**:
608 602645. https://doi.org/10.3389/fpls.2020.602645
609 Ferreyra ML, Rius SP, Casati P. 2012. Flavonoids: biosynthesis, biological functions, and biotechnological
610 applications. *Frontiers of Plant Science* **3**: 222. https://doi.org/10.3389/fpls.2012.00222
611 Fogacci F, Rizzo M, Krogager C, Kennedy C, Georges CM. 2020. Safety evaluation of α-lipoic acid
612 supplementation: a systematic review and meta-analysis of randomized placebo-controlled clinical
613 studies. *Antioxidants (Basel)* **9(10)**: 1-34. https://doi.org/10.3390/antiox9101011
614 Foyer CH, Lam HM, Nguyen HT, Siddique KH, Varshney RK, Colmer TD, Considine MJ. 2016. Neglecting
615 legumes has compromised human health and sustainable food production. *Nature Plants* **2(8)**: 1-10.
616 https://doi.org/10.1038/nplants.2016.112.
617 Giannopolitis CN, Ries SK. 1977. Superoxide dismutases: I Occurrence in higher plants. *Plant Physiology* **59(2)**:
618 309-314. https://doi.org/10.1104/pp.59.2.309
619 Gorcek Z, Erdal S. 2015. Lipoic acid mitigates oxidative stress and recovers metabolic distortions in salt-stressed
620 wheat seedlings by modulating ion homeostasis, the osmo-regulator level and antioxidant system. *Journal of*
621 *the Science of Food and Agriculture* **95(14)**: 2811-2817. https://doi.org/10.1002/jsfa.7020
622 Grieve CM, Grattan SR. 1983. Rapid assay for determination of water-soluble quaternary ammonium
623 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

Hasanuzzaman M, Raihan MRH, Masud AAC, Rahman K, Nowroz F, Rahman M, Fujita M. 2021. Regulation of reactive oxygen species and antioxidant defense in plants under salinity. *International Journal of Molecular Sciences*, **22(17)**, 9326. doi: 10.3390/ijms22179326

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 Journal 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

Irshad MK, Ibrahim M, Noman A, Shang J, Mahmood A, Mubashir M, Khoo KS, Ng HS, Show PL. 2022. Elucidating the impact of goethite-modified biochar on arsenic mobility, bioaccumulation in paddy rice (*Oryza sativa* L.) along with soil enzyme activities. *Process Safety and Environmental Protection* ***160*****:** 958-967. https://doi.org/10.1016/j.psep.2022.02.069

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.

661 Khalid MF, Hussain S, Ahmad S, Ejaz S, Zakir I, Ali MA, Anjum MA. 2019. Impacts of abiotic stresses on growth
662 and development of plants. In *Plant Tolerance to Environmental Stress* CRC Press, 1-8.
663 http://dx.doi.org/10.1201/9780203705315-1
664 Krol A, Amarowicz R, Weidner S. 2014. Changes in the composition of phenolic compounds and antioxidant
665 properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of long-term drought stress. *Acta*
666 *Physiologiae Plantarum* **36**: 1491-1499. http://doi.org/10.1007/s11738-014-1526-8
667 Kumar S, Korra T, Thakur R, Arutselvan R, Kashyap AS, Nehela Y, Keswani C. 2023. Role of plant secondary
668 metabolites in defence and transcriptional regulation in response to biotic stress. *Plant Stress*, 100154.
669 https://doi.org/10.1016/j.stress.2023.100154
670 Li N, Han X, Feng D, Yuan D, Huang LJ. 2019. Signaling crosstalk between salicylic acid and ethylene/jasmonate
671 in plant defense: do we understand what they are whispering? *International Journal of Molecular Sciences*
672 **20(3)**: 671. https://doi.org/10.3390/ijms20030671
673 Liang X, Zhang T, Lu X, Ellsworth DS, Bassirirad H, You C, Ye Q. 2020. Global response patterns of plant
674 photosynthesis to nitrogen addition: A meta-analysis. *Global Change Biology* **26(6)**: 3585-3600.
675 https://doi.org/10.1111/gcb.15071
676 Lindstrom K, Mousavi SA. 2020. Effectiveness of nitrogen fixation in rhizobia. *Microbial Biotechnology* **13(5)**:
677 1314-1335. https://doi.org10.1111/1751-7915.13517
678 Liu F, Andersen MN, Jacobsen SE, Jensen CR. 2005. Stomatal control and water use efficiency of soybean (*Glycine*
679 *max* L.) during progressive soil drying. *Environmental and Experimental Botany* **54(1)**: 33-40.
680 https://doi.org/10.1016/j.envexpbot.2004.05.002
681 Ma J, Aqeel M, Khalid N, Nazir A, Alzuaibr FM, Al-Mushhin AA, Hakami O, Iqbal MF, Chen F, Alamri S,
682 Hashem M, Noman, A. 2022. Effects of microplastics on growth and metabolism of rice (*Oryza sativa*
683 L.). *Chemosphere* ***307*****:** 135749. https://doi.org/10.1016/j.chemosphere.2022.135749
684 Majumdar R, Barchi B, Turlapati SA, Gagne M, Minocha R, Long S, Minocha SC. 2016. Glutamate ornithine,
685 arginine, proline, and polyamine metabolic interactions: the pathway is regulated at the post-transcriptional
686 level. *Frontiers of Plant Science* **7**: 1-17. https://doi.org/10.3389/fpls.2016.00078
687 Meena M, Divyanshu K, Kumar S, Swapnil P, Zehra A, Shukla V, Upadhyay RS. 2019. Regulation of L-proline
688 biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable
689 environmental conditions. *Heliyon*, **5(12)**. doi: 10.1016/j.heliyon. 2019.e02952
690 Mian HN, Mahmood S, Akram S, Ali Q, Azeem M. 2021. Evaluation of drought tolerance potential in mungbean
691 *Vigna radiata* (L.) R. Wilczek germ plasm resources: growth pigmentation and oxidative defense mechanism.
692 *Fresenius Environmental Bulletin* **30(8)**:10313-10325.
693 Mohammad-khani N, Heidari R. 2008. Water stress induced by polyethylene glycol 6000 and sodium. *Pakistan*
694 *Journal of Biological Sciences* **2(1)**: 92-97. https://doi.org/10.3923/pjbs.2008.92.97
695 Moini H, Packer L, Saris NEL. 2002. Antioxidant and prooxidant activities of α-lipoic acid and dihydrolipoic acid.
696 *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 MF, 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

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

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

Rajput VD, Harish SRK, Verma KK, Sharma L, Quiroz-Figueroa FR, Mandzhieva S. 2021. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. *Biology* **10(4)**, 267. doi: 10.3390/biology10040267

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

Saeed F, Kausar A, Ali Q, Akhter N, Tehseen S. 2023. Impact of combined glutathione and Zn application for seed priming in ameliorating the adverse effects of water stress on maize seed germination attributes, metabolite levels, and seedling vigor. *Gesunde Pflanzen* 1-22. https://doi.org/10.1007/s10343-023-00831-6

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

Shahid S, Ali Q, Ali S, Al-Misned FA, Maqbool S. 2022. Water deficit stress tolerance potential of newly developed wheat genotypes for better yield based on agronomic traits and stress tolerance indices: Physio-biochemical responses, lipid peroxidation and antioxidative defense mechanism. *Plants **11*(3)**: 466. https://doi.org/10.3390/plants11030466

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 JS, 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.

769 Shinde S, Villamor JG, Lin W, Sharma S, Verslues PE. 2016. Proline coordination with fatty acid synthesis and
770 redox metabolism of chloroplast and mitochondria. *Plant Physiology* **172(2):** 1074-1088.
771 https://doi.org/10.1104/pp.16.01097
772 Singh CM, Singh AK, Mishra SB, Pandey A. 2016. Generation mean analysis to estimate the genetic parameters for
773 yield improvement and inheritance of seed colour and lusture in mungbean [*Vigna radiata* (L.)
774 Wilczek]. *Legume Research An International Journal* **39**: 1-18. http://doi.org/
775 http://dx.doi.org/10.18805/lr.v0iOF.10762
776 Singh P, Pandey B, Pratap A, Gyaneshwari U, Nair RM, Mishra AK, Singh CM. 2022. Genetic and genomics
777 resources of cross-species *vigna* gene pools for improving biotic stress resistance in mungbean (*Vigna radiata*
778 L. Wilczek). *Agronomy* **12(12):** 3000. https://doi.org/10.3390/agronomy12123000
779 Tabassum MA, Zhu GL, Hafeez A, Wahid MA, Shaban M, Li Y. 2016. Influence of leaf vein density and thickness
780 on hydraulic conductance and photosynthesis in rice (*Oryza sativa* L.) during water stress. *Science Reports* **6(1)**:
781 36894. https://doi.org/10.1038/srep36894
782 Tahir A, Qadir M, Saif R, Sattar S, Tahir S. 2019. Morphological and yield response of pulses against drought
783 stress: A review. *Turkish Journal of Agriculture-Food Science and Technology* **7(2)**: 202-208.
784 https://doi.org/10.24925/turjaf.v7i2.202-208.2178
785 Terzi R, Saruhan GN, Guven FG, Kadioglu A. 2018. Alpha lipoic acid treatment induces the antioxidant system and
786 ameliorates lipid peroxidation in maize seedlings under osmotic stress. *Archives of Biological Sciences* **70(3)**:
787 503-511. https://doi.org/10.2298/ABS171218011T
788 Turk H, Erdal S, Karayel U, Dumlupinar R. 2018. Attenuation of lead toxicity by promotion of tolerance mechanism
789 in wheat roots by lipoic acid. *Cereal Research Communications* **46**: 424-435. http://doi.org/
790 10.1556/0806.46.2018.020
791 Uddin S, Ullah S, Nafees M. 2021. Effect of seed priming on growth and performance of *Vigna radiata* L. under
792 induced drought stress. *Journal of Agriculture and Food Research* **4**: 100140.
793 https://doi.org/10.1016/j.jafr.2021.100140
794 Uppalwar SV, Garg V, Dutt R. 2021. Seeds of mung bean (*Vigna radiata* (L.) R. wilczek): taxonomy,
795 phytochemistry, medicinal uses and pharmacology. *Current Bioactive Compounds* **17(3)**: 220-233.
796 https://doi.org/10.2174/1573407216999200529114608.
797 Urmi TA, Islam MM, Zumur KN, Abedin MA, Haque MM, Siddiqui MH, Hoque MA. 2023. Combined effect of
798 salicylic acid and proline mitigates drought stress in rice (*Oryza sativa* L.) through the modulation of
799 physiological attributes and antioxidant enzymes. *Antioxidants* **12(7):** 1438.
800 https://doi.org/10.3390/antiox12071438
801 Velikova V, Yordanov I, Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean
802 plants: protective role of exogenous polyamines. *Plant Science* **151(1)**: 59-66. https://doi.org/10.1016/S0168-
803 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

Yadav M, Gupta P, Seth CS. 2022. Foliar application of α-lipoic acid attenuates cadmium toxicity on photosynthetic pigments and nitrogen metabolism in *Solanum lycopersicum* L. *Acta Physiologiae Plantarum* **44(11)**, 1-10 https://doi.org/10.1007/s11738-022-03445-z.

Youssef MH, Raafat A, El-Yazied AA, 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 reduced irrigation.

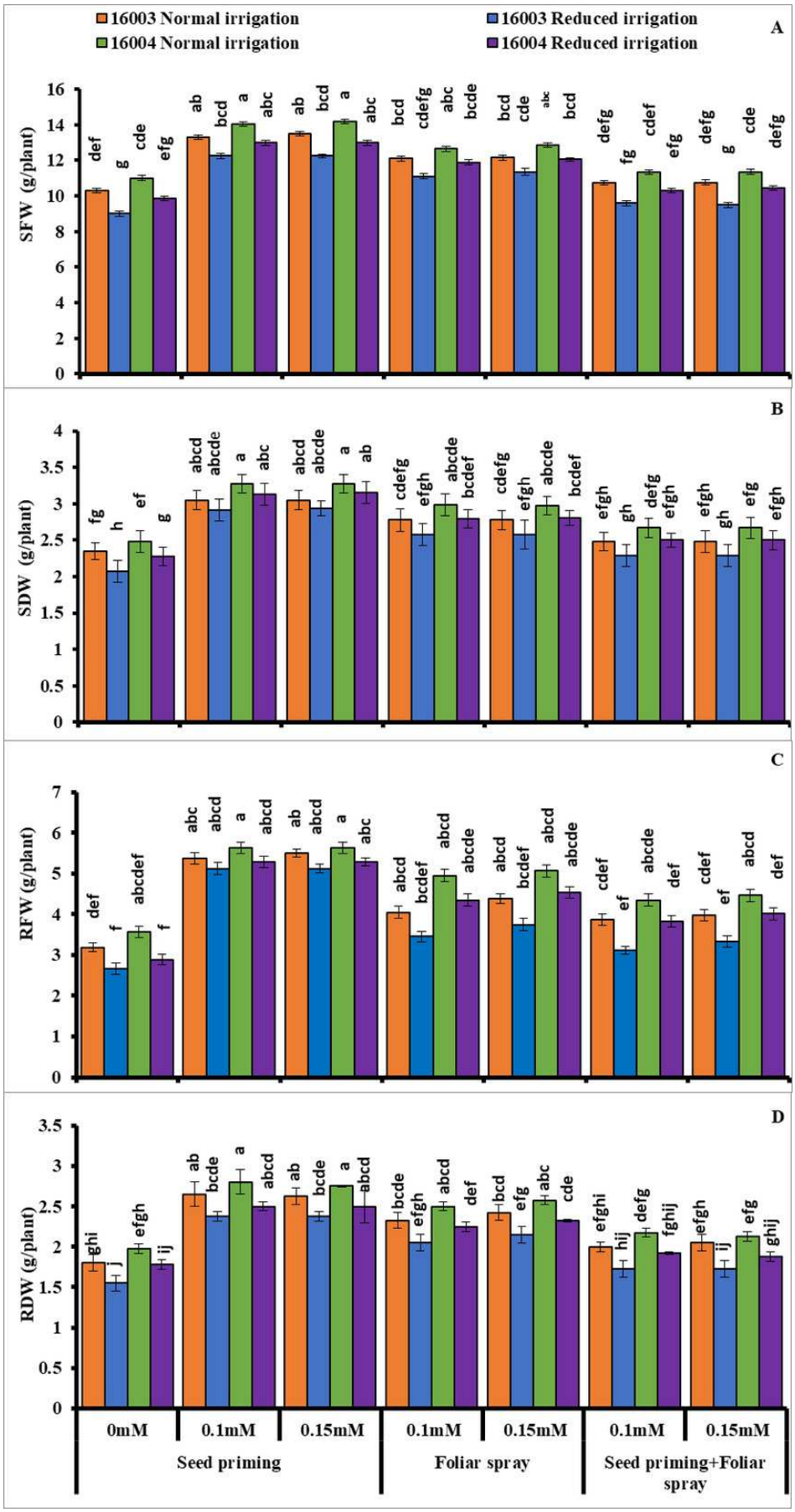

16003 Normal irrigation
16003 Reduced irrigation
16004 Normal irrigation
16004 Reduced irrigation
A
SFW (g/plant)
B
SDW (g/plant)
C
RFW (g/plant)
D
RDW (g/plant)
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

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 reduced irrigation.

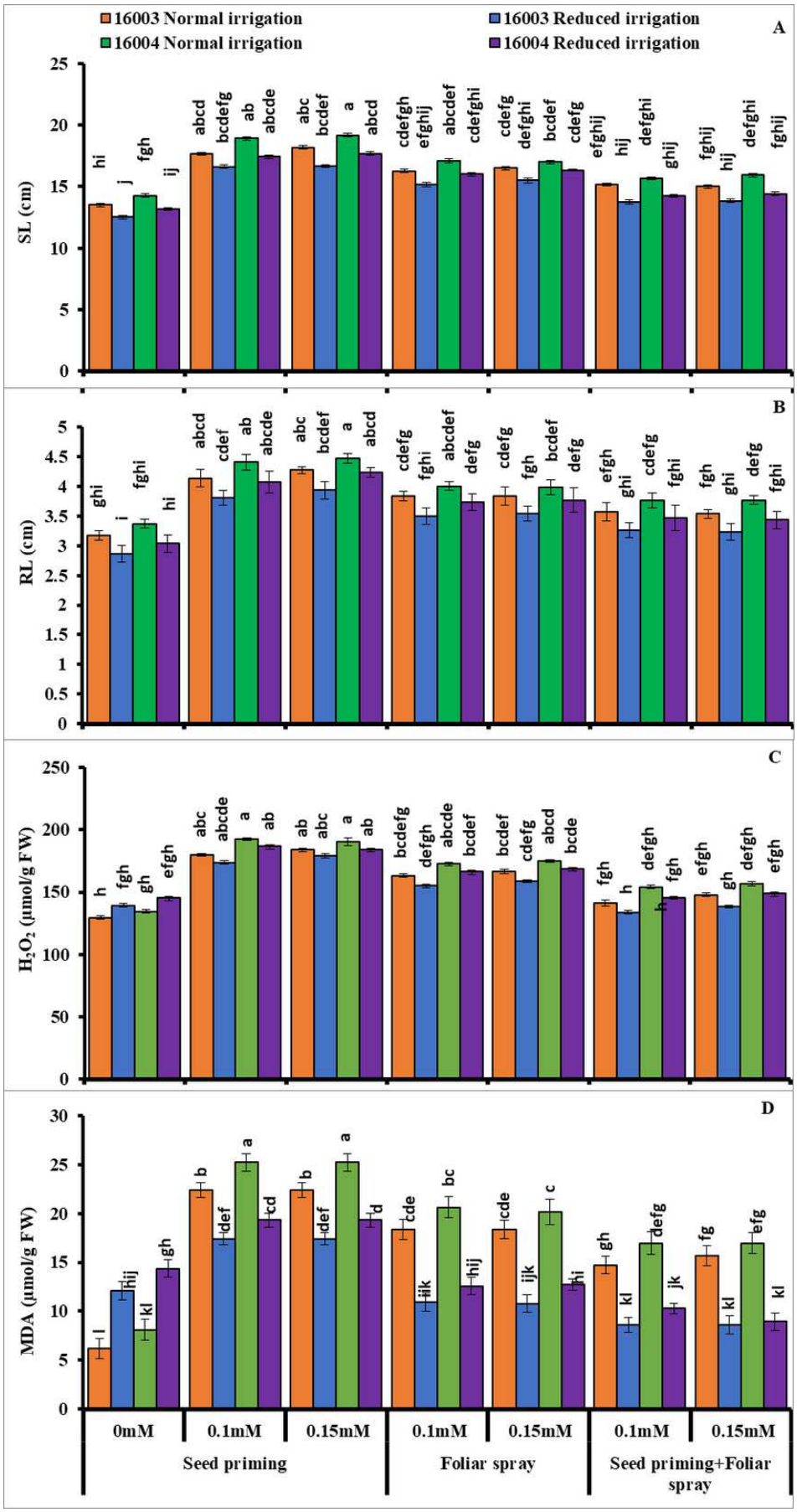

16003 Normal irrigation
16003 Reduced irrigation
16004 Normal irrigation
16004 Reduced irrigation
A
SL (cm)
B
RL (cm)
C
H2O2 (µmol/g FW)
D
MDA (µmol/g FW)
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

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 reduced irrigation

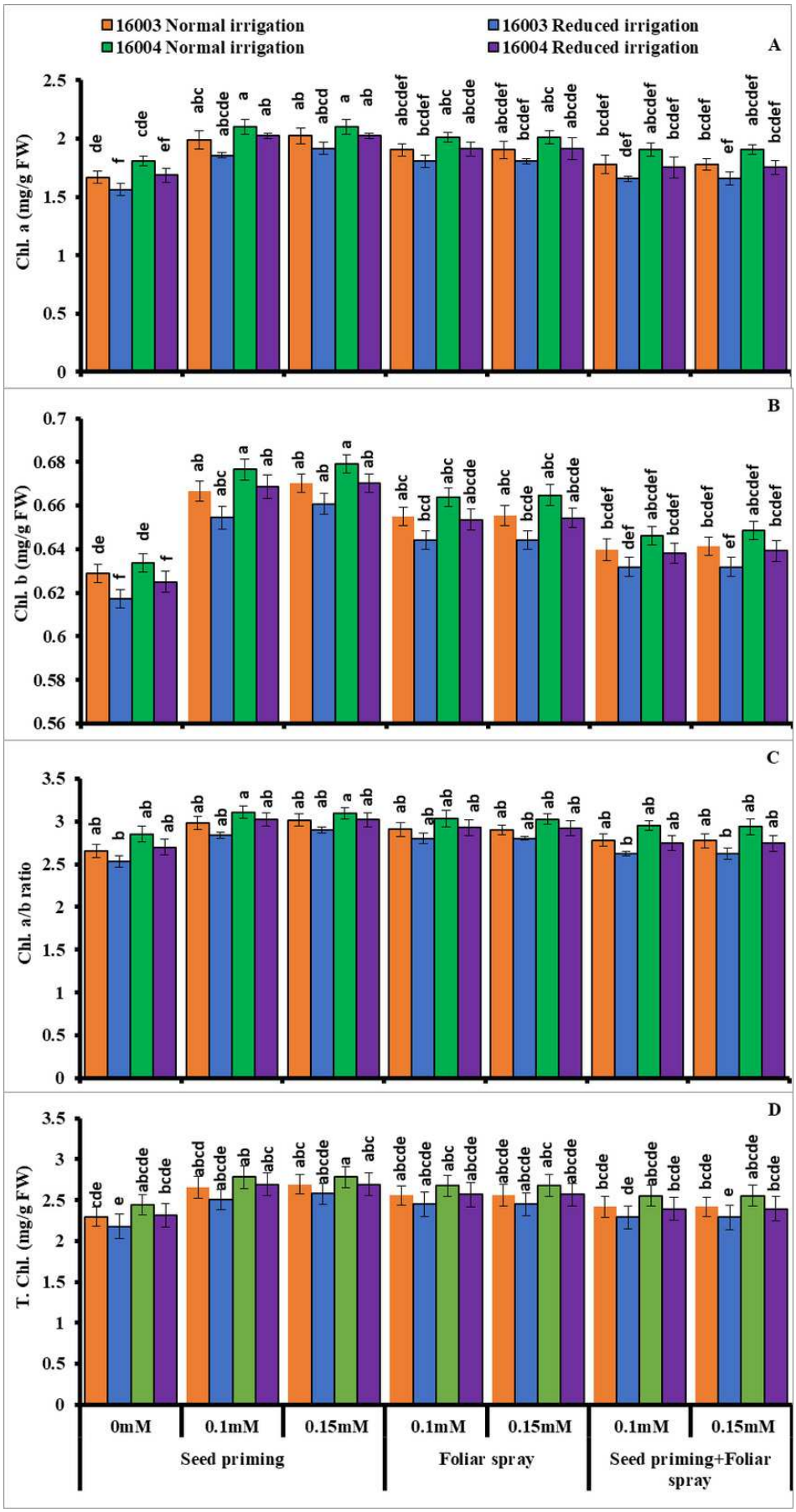

16003 Normal irrigation
16003 Reduced irrigation
16004 Normal irrigation
16004 Reduced irrigation
A
Chl. a (mg/g FW)
B
Chl. b (mg/g FW)
C
Chl. a/b ratio
D
T. Chl. (mg/g FW)
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

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 reduced irrigation.

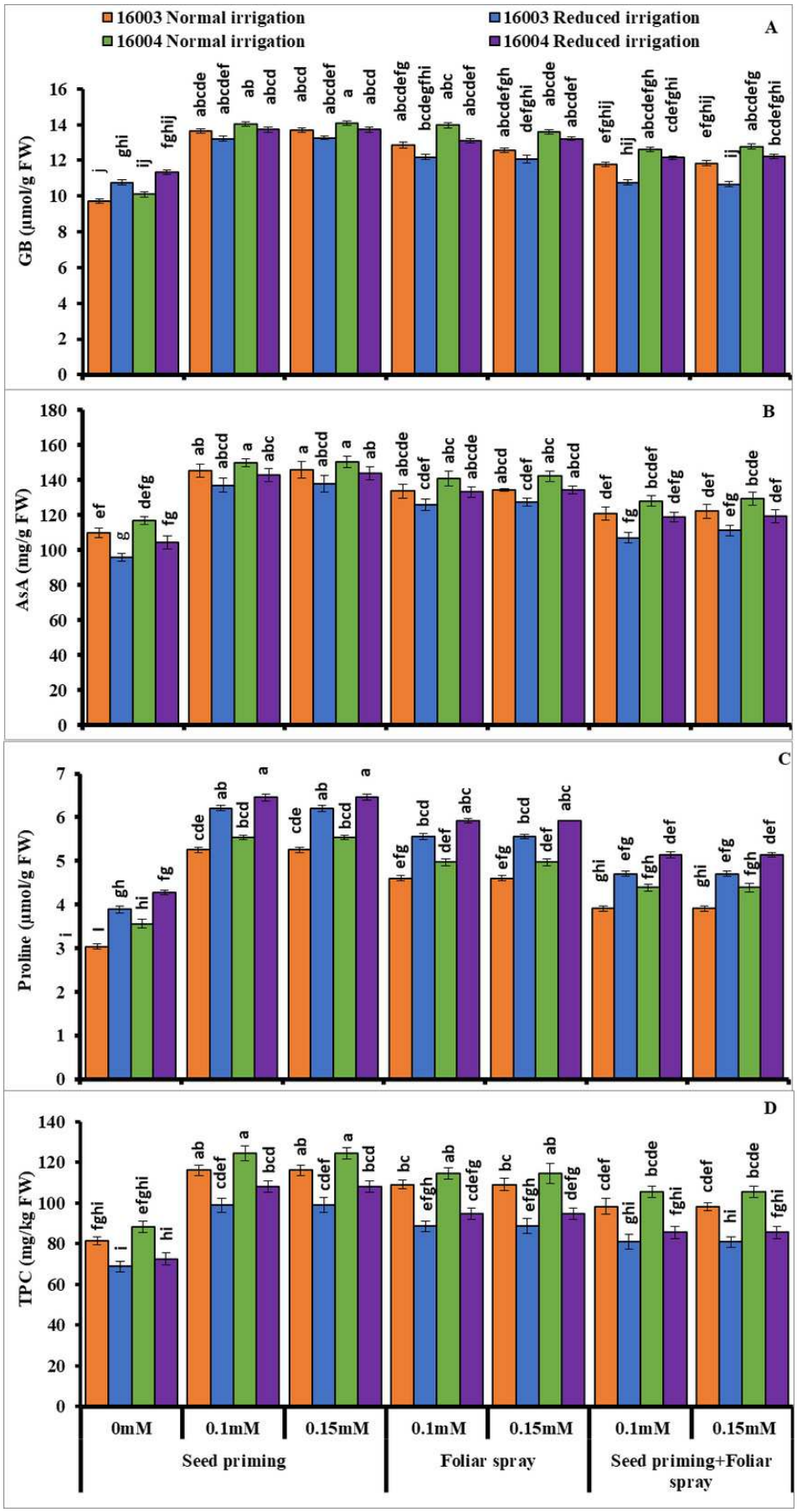

16003 Normal irrigation
16003 Reduced irrigation
16004 Normal irrigation
16004 Reduced irrigation
A
GB (µmol/g FW)
B
AsA (mg/g FW)
C
Proline (µmol/g FW)
D
TPC (mg/kg FW)
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

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 reduced irrigation.

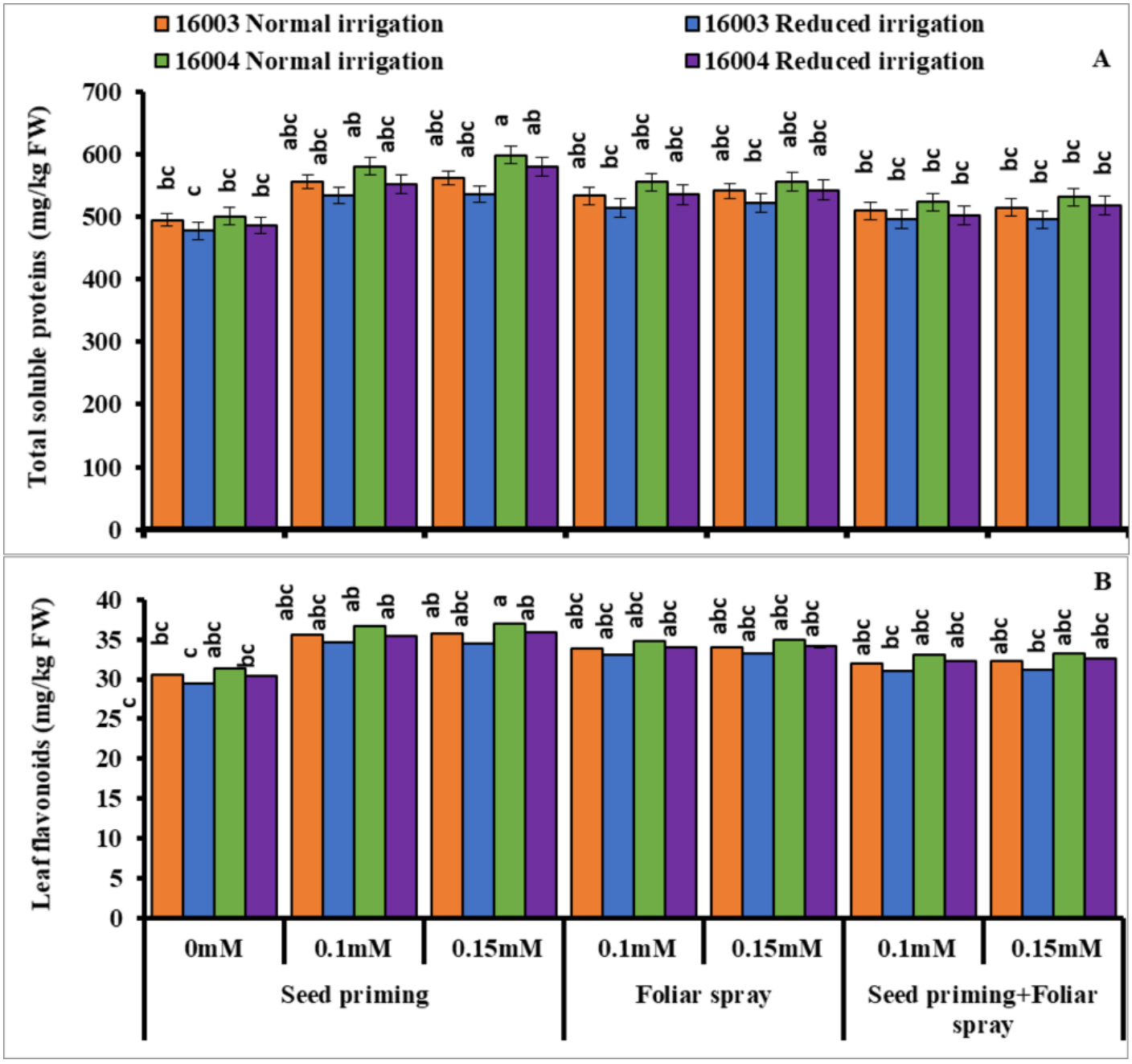


Figure 6

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 reduced irrigation.

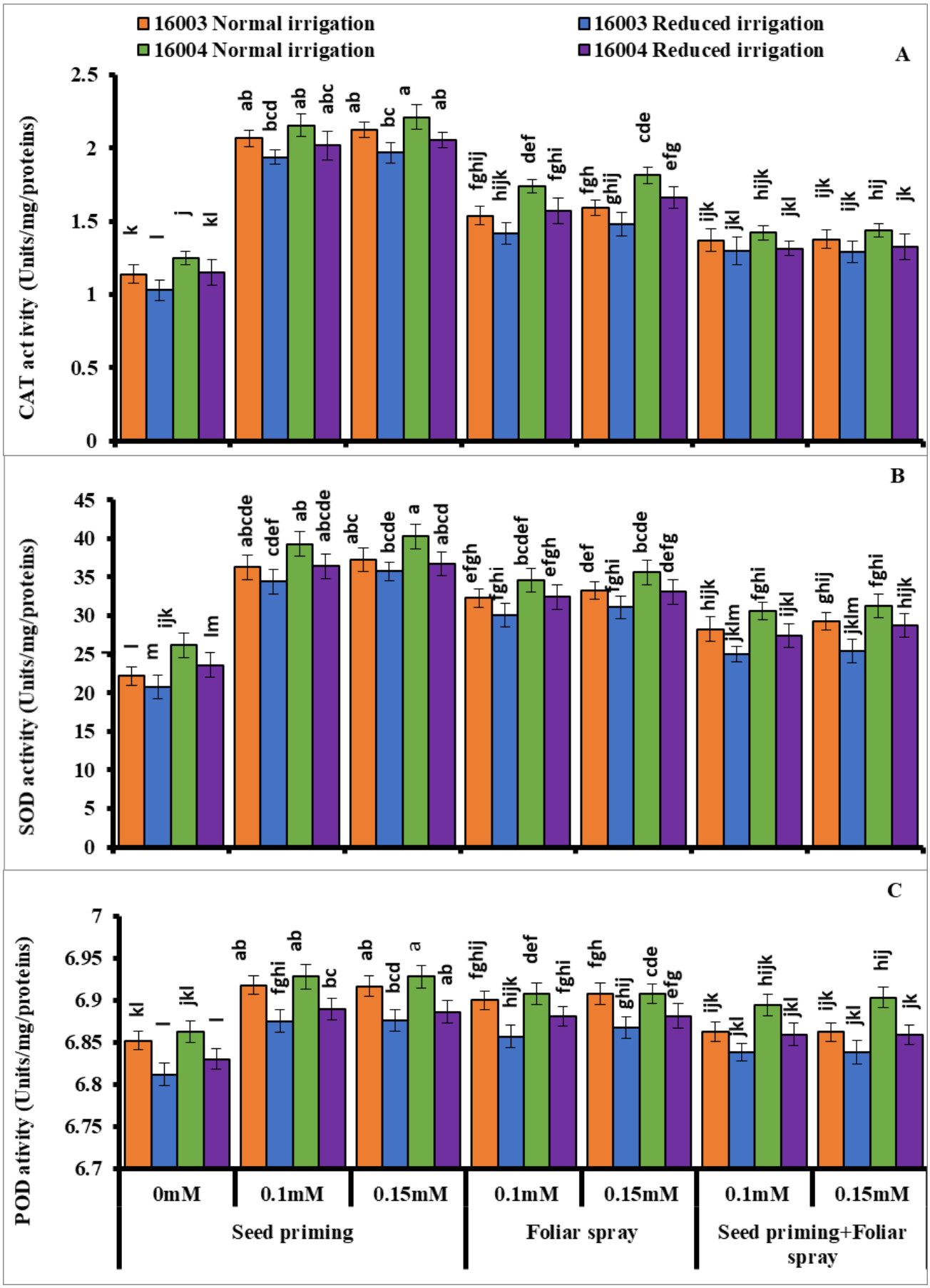

16003 Normal irrigation
16003 Reduced irrigation
16004 Normal irrigation
16004 Reduced irrigation
A
CAT activity (Units/mg/proteins)
B
SOD activity (Units/mg/proteins)
C
POD ativity (Units/mg/proteins)
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

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 reduced irrigation.

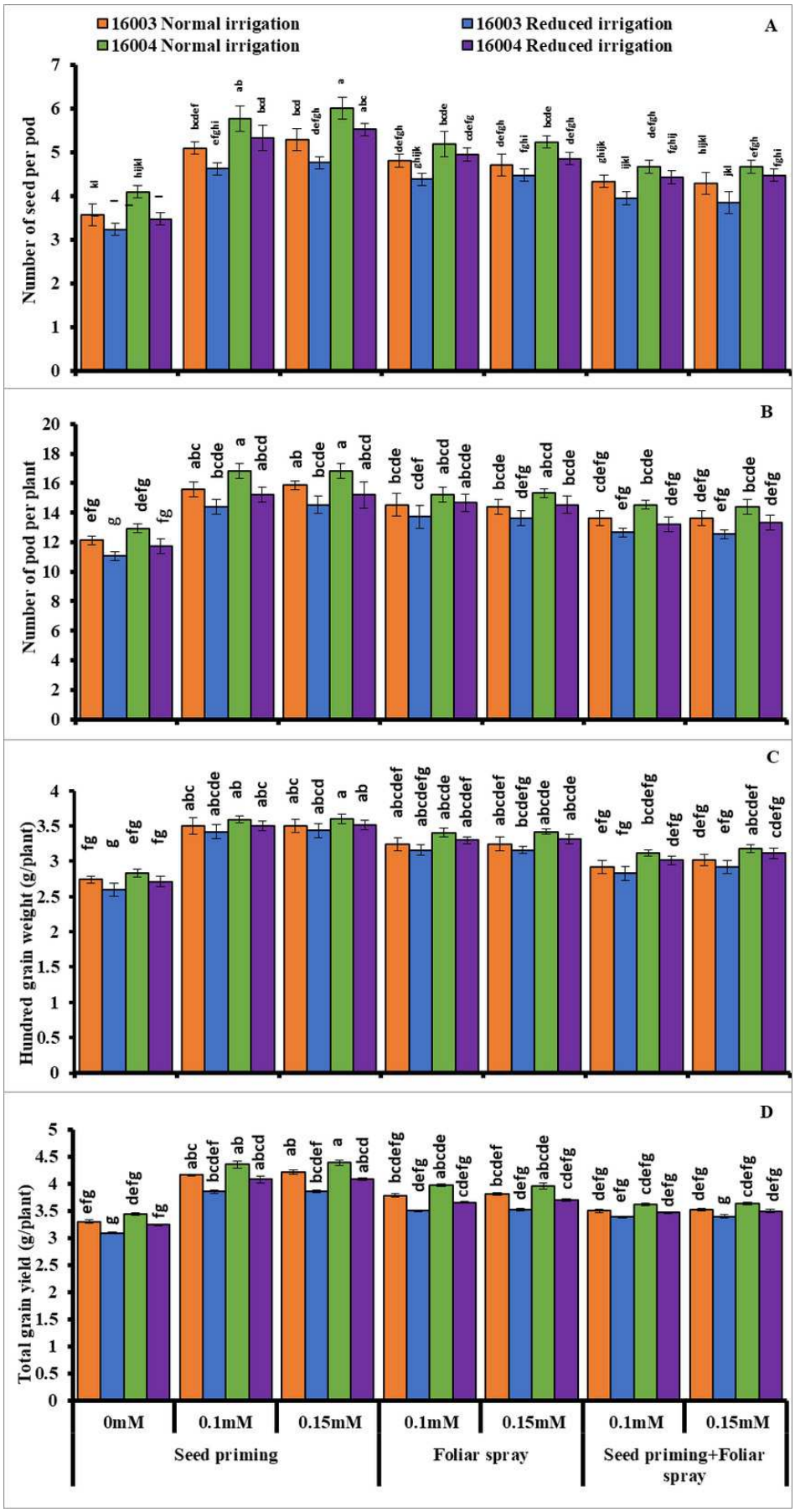

16003 Normal irrigation
16003 Reduced irrigation
16004 Normal irrigation
16004 Reduced irrigation
A
Number of seed per pod
B
Number of pod per plant
C
Hundred grain weight (g/plant)
D
Total grain yield (g/plant)
0mM
0.1mM
0.15mM
0.1mM
0.15mM
0.1mM
0.15mM
Seed priming
Foliar spray
Seed priming+Foliar spray

Figure 8

Heatmap histogram correlation between different studied attributes of mung bean line (16003) fertigated with different levels of lipoic acid

Priming [(Control 0 mM (1), 0.1 mM (2), 0.15 mM (3), Stress 0 mM (4), 0.1 mM (5), 0.15 mM (6)], Foliar [Control 0.1 mM (7), 0,15 mM (8), Stress (0 mM (9), 0.1 mM (10), 0.15 mM (11)] and Priming+ Foliar [Control 0.1 mM (12), 0.15 mM (13), Stress 0 mM (14), 0.1 mM (15), 0.15 mM (16)] when grown under normal irrigation and reduced irrigation.

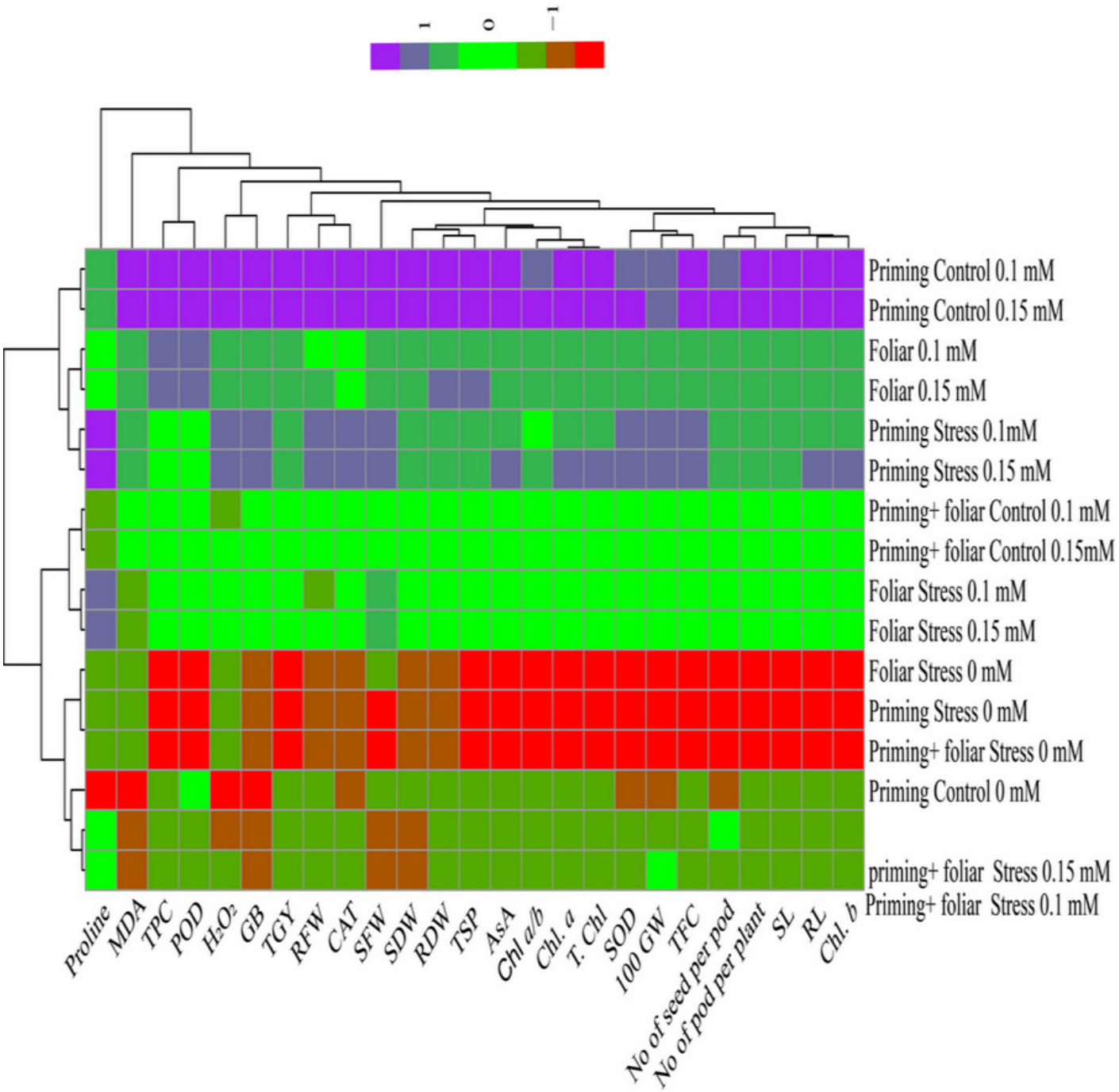
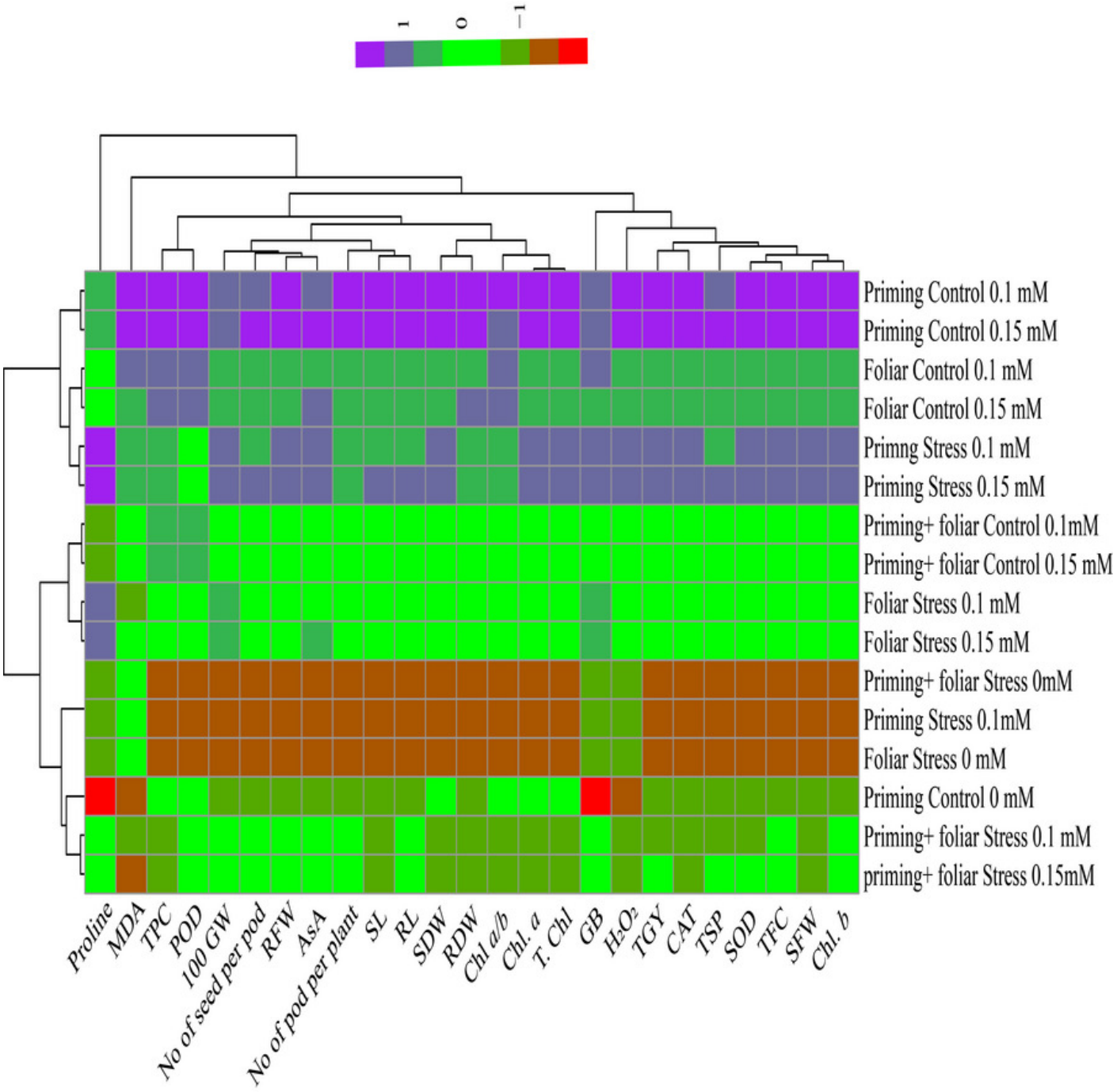

1
0
-1
Priming Control 0.1 mM
Priming Control 0.15 mM
Foliar 0.1 mM
Foliar 0.15 mM
Priming Stress 0.1mM
Priming Stress 0.15 mM
Priming+ foliar Control 0.1 mM
Priming+ foliar Control 0.15mM
Foliar Stress 0.1 mM
Foliar Stress 0.15 mM
Foliar Stress 0 mM
Priming Stress 0 mM
Priming+ foliar Stress 0 mM
Priming Control 0 mM
priming+ foliar Stress 0.15 mM
Priming+ foliar Stress 0.1 mM
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 line (16004) fertigated with different levels of lipoic acid

Priming [(Control 0 mM (1), 0.1 mM (2), 0.15 mM (3), Stress 0 mM (4), 0.1 mM (5), 0.15 mM (6)], Foliar [Control 0.1 mM (7), 0,15 mM (8), Stress (0 mM (9), 0.1 mM (10), 0.15 mM (11)] and Priming+ Foliar [Control 0.1 mM (12), 0.15 mM (13), Stress 0 mM (14), 0.1 mM (15), 0.15 mM (16)] when grown under normal irrigation and reduced irrigation.


1
0
-1
Priming Control 0.1 mM
Priming Control 0.15 mM
Foliar Control 0.1 mM
Foliar Control 0.15 mM
Primng Stress 0.1 mM
Priming Stress 0.15 mM
Priming+ foliar Control 0.1mM
Priming+ foliar Control 0.15 mM
Foliar Stress 0.1 mM
Foliar Stress 0.15 mM
Priming+ foliar Stress 0mM
Priming Stress 0.1mM
Foliar Stress 0 mM
Priming Control 0 mM
Priming+ foliar Stress 0.1 mM
priming+ foliar Stress 0.15mM
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
H2O2
TGY
CAT
TSP
SOD
TFC
SFW
Chl. b