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Original article

# Physiological, biochemical and molecular evaluation of mungbean genotypes for agronomical yield under drought and salinity stresses in the presence of humic acid

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## ABSTRACT

Drought and salinity are potential threats in arid and semi arid regions. The current study was conducted with objective to optimize the production of different exotic genotypes of mungbean (NM-121-25, Chakwal M-6, DM-3 and PRI-Mung-2018) under drought and salinity stresses using humic acid in field experiments. One year tri-replicate field experiment was performed in RCBD using three factorial arrangement and effects of humic acid ( $60 \text{ kg ha}^{-1}$ ) were evaluated at physiological, biochemical, molecular and agronomical level under individual and integrated applications of drought (no irrigation till 15 days) and salinity ( $\text{EC } 6.4 \text{ dSM}^{-1}$ ). Data for physiological parameters (total chlorophyll, photosynthesis rate, stomatal conductance, transpiration rate and membrane damage), antioxidant enzymes (superoxide dismutase, catalase, peroxidase) and proline were collected on weekly basis since after the initiation of drought and salinity stresses. However data for agronomic characteristics (plant height, branches  $\text{plant}^{-1}$ , LAI, pods  $\text{plant}^{-1}$ , pod length and hundred seed weight) and grain carbohydrate content were collected after harvesting, while sampling for drought (*VrDREB2A*, *VrbZIP17* and *VrHsfA6a*) and salinity (*VrWRKY73*, *VrUBC1* and *VrNHX1*) related genes expression study was done after plants attained seedling stage. Under both individual and integrated applications of drought and salinity, all genotypes showed significant ( $p \leq 0.05$ ) increase in all traits excluding Cell membrane damage and proline during humic acid application. Likewise, genes expression revealed statistically distinct ( $p \leq 0.05$ ) up-regulation under humic acid treatment as compared to no humic acid treatment during both individual and integrated applications of drought and salinity. The genotype PRI-Mung-2018 recorded noteworthy performance during study. Moreover correlation and PCA analysis revealed that ultimate agronomical yield due to humic acid is an outcome of interconnection of physiological and biochemical parameters.

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**Abbreviations:** ROS, Reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; MD, membrane damage; RCBD, randomized complete block design; HA0, the presence HA of humic acid; Pn, photosynthesis rate; Gs, stomatal conductance; Tr, transpiration rate; PH, plant height; PL, pod length; LAI, leaf area index; BPP, branches per plant; PPP, pods per plant; HSW, hundred seed weight; GCC, Grain carbohydrate content.

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## 1. Introduction

Mungbean (*Vigna radiata* L.) is an eminent short duration legume crop whose cultivation is expanding across the sphere owe to its antioxidants, high protein, fibers and nutrients profile. Mungbean generally grows in rain-fed areas having low humidity, with moderate rainfall and high temperatures  $27\text{--}30^\circ\text{C}$  (Alghabari, 2020). Therefore, its various developmental phases face drought stress. Although it is speculated that mungbean can survive during drought conditions, however the response of different cultivars is variable depending upon crop stage of growth, extent and duration of abiotic stresses (Singh et al., 2021).

Overall all kinds of abiotic stresses hamper the production of mungbean, however salinity and drought are potential constraints for all stages of growth and developmental of plants under arid and

**Table 1**

List of primers used for qRT-PCR analysis of genes.

| Gene     | Forward                  | Reverse                 |
|----------|--------------------------|-------------------------|
| VrDREB2A | CTGCTCTTGCTTATGATGAA     | ATGTAGTGGTAGTAGTAGTAGT  |
| VrbZIP17 | GGCATCATCATCTCCATG       | GAGTTTGAGGTCGTTC        |
| VrHsfA6a | GCTTGCTATGAACATGGAGGA    | TCATTATCATCATTTTCCAATGC |
| VrWRKY73 | CGCAGAAGTGTGTGGTGA       | GTGGTGTGGTGTATGCAG      |
| VrUBC1   | ATGGCCTCTAAGCGGATTCTGAAG | CTAGCCCATTCATCTCTGACTCC |
| VrNHX1   | GCTGTATATTGGAAGGCACTCT   | GGCCACGCGTCGACTAGTAC    |
| VrActin  | TCCACGAGACAACATATAACT    | TCCTTGCTCATCTATCAG      |

semiarid conditions (Sehrawat et al., 2020; Singh et al., 2021). Exposure of plant to these changes results in the change of physiological, biochemical and metabolic processes (Shah et al., 2018). Drought and salinity tolerance ability of mungbean can be estimated by measuring various morphological, physiological and biochemical parameters (HanumanthaRao et al., 2016; Bangar et al., 2019). Many studies reported significant variation in morphological, physiological responses of mungbean for tolerance against drought and salinity stresses (Naresh et al., 2013; HanumanthaRao et al., 2016; Raina et al., 2019; Sehrawat et al., 2020). Besides, salinity stress is not only the problem of irrigated area but also an intensive problem of arid and semi-arid regions. Similar to drought, salinity affects physio-morphic and biochemical attributes of mungbean (Sehrawat et al., 2020).

In this regard to relieve the plants from the effects of abiotic stresses agricultural practices needs the augmentation of soil conditioner such a humic acid that improves physical, chemical and structural properties of soil. Therefore, sustainable agriculture in prevailing conditions of abiotic stresses not only depends on proper inorganic fertilizers, but also on organic supplements containing biologically active compounds (Alghabari, 2020). Humic acid is an important plant biostimulant that improves soil water holding capacity in addition to nutrients uptake (Shah et al., 2018).

Furthermore, drought and salinity result in the generation of reactive oxygen species (ROS) whose excessive accumulation damages cellular constituents such as chloroplast, homeostatic proteins, nucleic acids and cell membrane (Pang et al., 2010; Shah et al., 2017; Farooq et al., 2019). Due to stress, the activities of various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) are inhibited, hence increasing level of ROS leads to membrane damage (MD) and programmed cell death (Apel and Hirt, 2004; Hasanuzzaman et al., 2012; Nair et al., 2012; Nair et al., 2019). Humic acid improves crop tolerance against drought and salinity due to activation of different biochemical and physiological activities, for instance improved stomatal conductance, and activation of the antioxidant enzymes such as SOD, CAT and POD to undermine the hazards of ROS (Abbas et al., 2022). It has tendency to reduce peroxidation and enhance the homeostatic protein contents by accelerating the uptake of essential nutrients, particularly when soil conditions are marginal (Dinçsoy and Sönmez, 2019).

Apart from this, the tools of functional of genomics revolutionized our understanding about gene interaction and expression during abiotic stress. This paved the way toward devising the better strategies for incorporating the abiotic stress tolerance. Many researchers have performed transcriptome based studies of various Vigna species (Liu et al., 2016; Butsayawarapat, et al., 2019). The genes such as VrDREB2A, VrbZIP17 and VrHsfA6a illustrate significant up-regulation in mungbean under the conditions of drought stress which authenticate their dynamic role in pathway of drought tolerance (Labbo et al., 2018; Li et al., 2018). Correspondingly, the genes such as VrWRKY73, VrUBC1 and VrNHX1 depict significant regulation in their expression when mungbean genotypes expose to the salinity stress that triggers various sort of osmotic changes and osmotic adjustments within plant (Srivastava et al.,

2018). Besides, HA regulates the gene expression (Shah et al., 2018), enhances the enzymatic activities that activate different plant physiological processes leading toward increased agronomic yield (Brown et al., 2014).

Saudi Arabia being located in arid climatic conditions, is a region of water scarcity having vast area comprising sandy soil with poor water holding capacity (Alghabari, 2020). Moreover, the processed Red Sea water is the sole source of irrigation that is augmenting the problem of salinity in the region in addition to drought. Additionally, mineral weathering due to high temperature is also aggravating the problem (Elhag, 2016). Mungbean being an important legume crop has dynamic importance in Saudi life style. Therefore, it is hypothesized that augmentation of soil management practices with supplementation of humic acid can be an effective tool in mitigating the devastating impacts of drought and salinity. In this perspective, current study aims to elucidate the impact of humic acid on molecular, physiological, biochemical and agronomic attributes of different exotic genotypes of mungbean under individual and integrated application of drought and salinity stresses.

## 2. Materials and methods

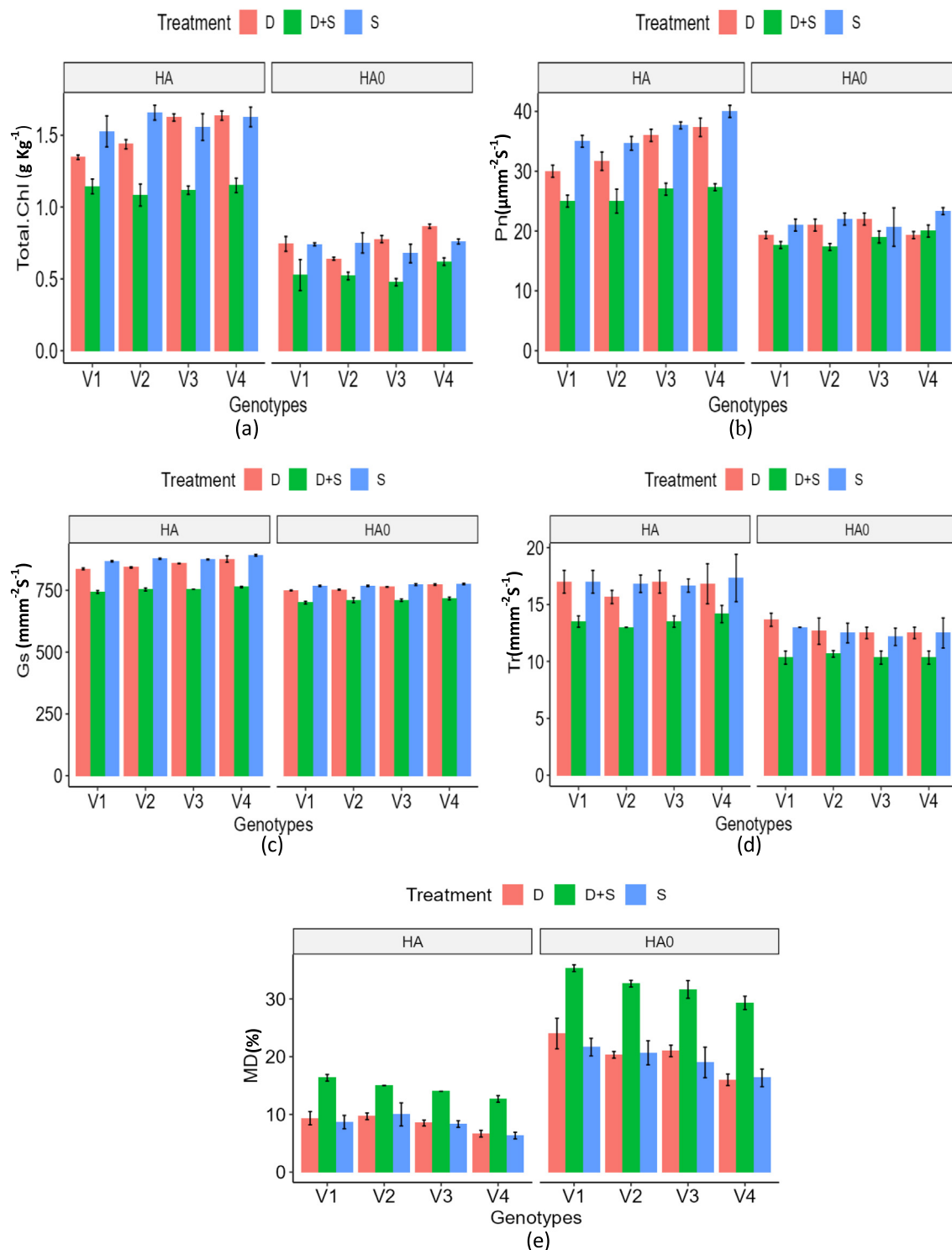
In the present study, four different mungbean cultivars viz; NM-121-25 (V1), Chakwal M-6 (V2), DM-3 (V3), PRI-Mung-2018 (V4) collected from Pakistan were evaluated in field experiment from October 2021 to March 2022 at King Abdulaziz university, Jeddah. The experimental site is characterized by an annual rainfall of 53 mm, humidity 50–60% and temperature 19–38 °C during growing season. The soil of the experimental site was loamy sand and moderately alkaline (PH 8.3). The study was conducted to evaluate the effect of humic acid (HA) on biochemical, physiological, agronomical and molecular parameters of mungbean genotypes under individual and integrated treatments of drought and salinity stresses using three factorial arrangements in randomized complete block design (RCBD).

### 2.1. Crop husbandry

Before sowing, soil was well ploughed and laddered. After this, 36–42 seeds M<sup>-2</sup> were sown at the depth of 4 cm using seed drill in 25 cm spaced rows having plant to plant distance of 10 cm. Moreover, plants were irrigated with normal water on daily basis using drip irrigation system till plants attained physiological maturity at vegetative stage. Fertilizer NPK (20:20:20) was applied by fertigation following the schedule used by Alghabari (2020) in three doses. In order to keep the crop healthy weeding was done after every 15 days.

### 2.2. Treatments application and data collection

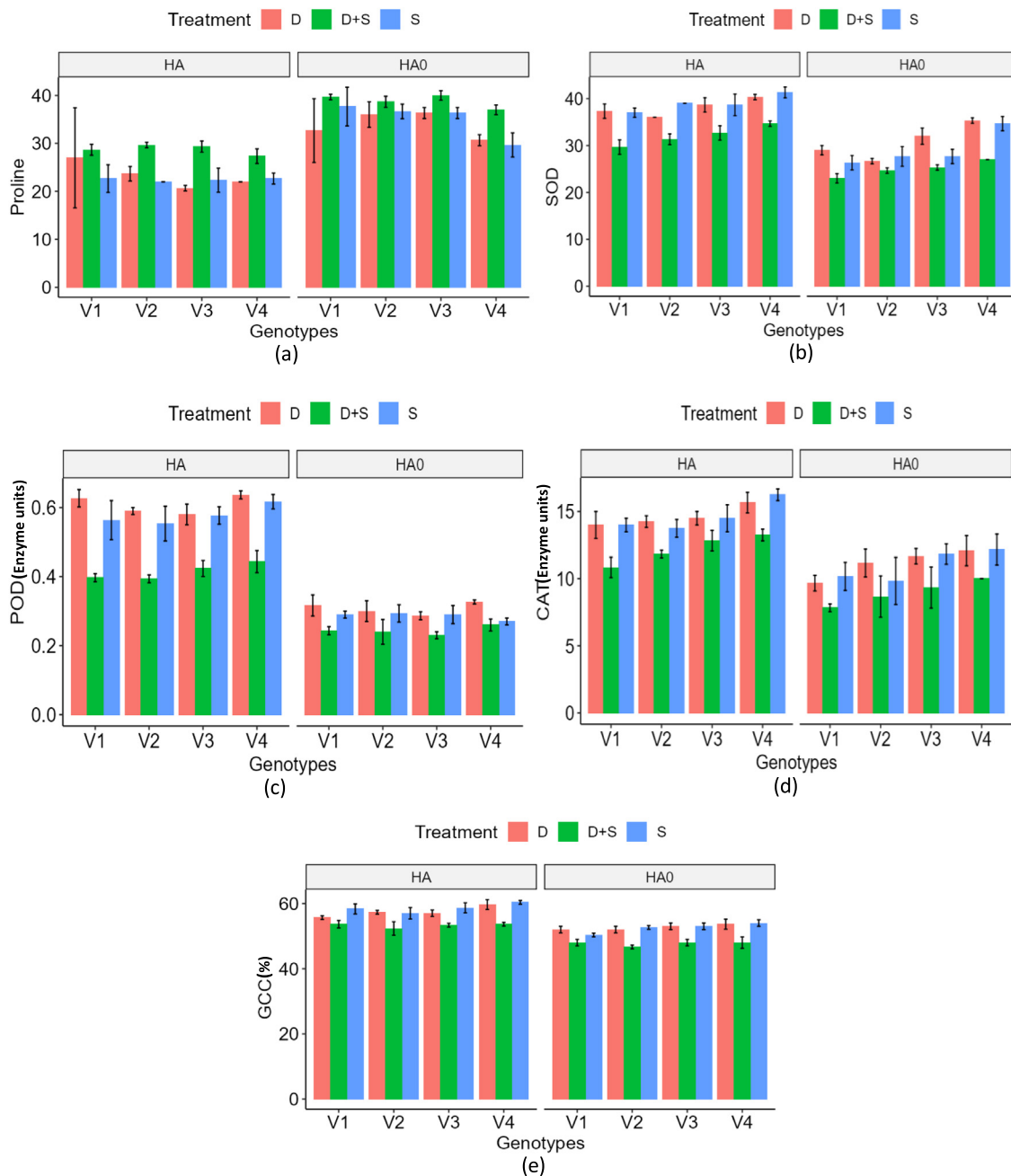
The effect of individual and integrated applications of drought and salinity treatment was tested in the absence (HA<sub>0</sub>) and presence (HA) of humic acid. Humic acid was applied in liquid



**Fig. 1.** Effect of individual and integrated treatments of drought and salinity on physiological characteristics of mungbean genotypes due to presence (HA) and absence (HA0) of humic acid.

phase at the rate of 60 kg ha<sup>-1</sup> in three equally divided doses as used by [Alghabari \(2020\)](#). First dose was implemented exactly after germination proceeded by two weeks later implementation of second dose, while third dose was implemented at the initiation of flowering. Drought stress was implemented according the schedule used by [Bangar et al. \(2019\)](#) as no irrigation till 15 days from vegetative stage and retained till plants attained reproductive

stage. Likewise, salinity stress was also implemented from vegetative stage till reproductive stage using saline water (EC 6.4 dSM<sup>-1</sup>) through drip irrigation system according to [Minhas et al. \(1990\)](#). Data regarding physiological traits, antioxidant enzymes and proline were collected on weekly basis since after the initiation of drought and salinity stresses. Data for agronomic characteristics were collected after harvesting. While sampling for drought and



**Fig. 2.** Effect of individual and integrated treatments of drought and salinity on biochemical parameters of mungbean genotypes due to presence (HA) and absence (HA0) of humic acid.

salinity related genes expression study was done after plants attained seedling stage.

### 2.3. Measurement of physiological traits

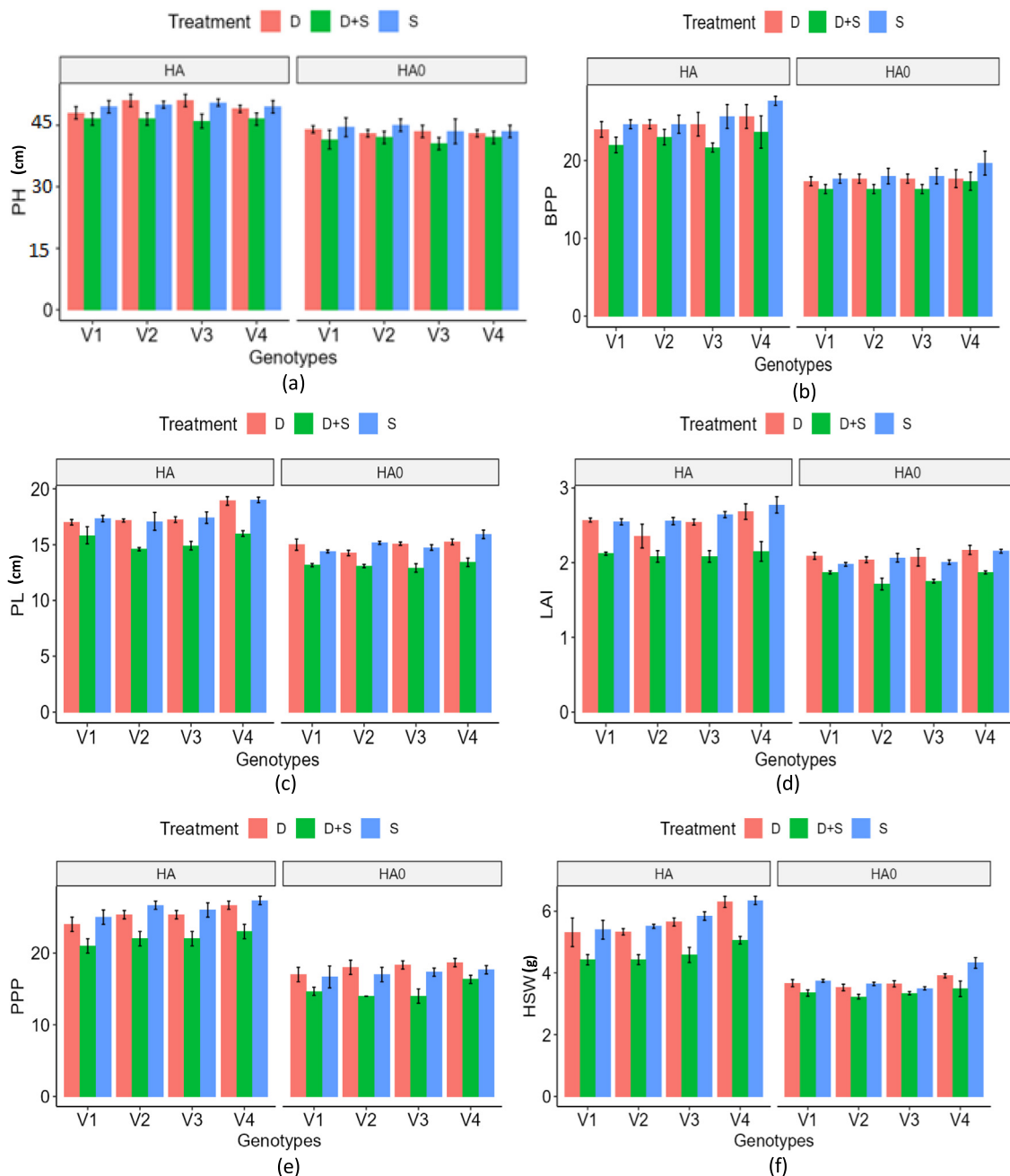
Physiological traits such as photosynthesis rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) were determined from fully expanded leaves between 8 am to 12 pm using device, CIRAS-3 updated version SC-1 (Amesbury, MA 01913, USA). Furthermore total chlorophyll (total chl) was recorded following the method opted by Mahmood et al. (2016). Cell membrane damage (MD) percentage was calculated following the methodology used by Alghabari et al. (2021). In this perspective 100 mg leaf pieces

were taken in two different tubes each with 20 mL deionized water. Afterward one tube was kept at 40 °C for half hour and its conductivity C1 was calculated, however second tube was maintained for 10 mins at 100 °C, and conductivity C2 was calculated. At the end, MD was determined using the formula  $[1 - (C1/C2)] \times 100$ .

### 2.4. Estimation of biochemical parameters

#### 2.4.1. Proline and antioxidant enzymes

The proline content was calculated from randomly selected leaf samples on the basis of proline-ninhydrin reactivity using UV-vis spectrophotometer (DeNovix, United States, Product No. DS-



**Fig. 3.** Effect of individual and integrated treatments of drought and salinity on agronomical traits of mungbean genotypes due to presence (HA) and absence (HA0) of humic acid.

11FX). On the other hand, antioxidant enzymes activities for instance SOD, CAT and POD were measured using available kits. The SOD activity was estimated with the help of SOD1 elisa kit (MyBioSource, United States, Product No. MBS283325) following the instructions given by manufacturer. The CAT activity was recorded following the protocol given by the manufacturer on catalase assay kit (MyBioSource, United States, Product No. MBS8243260). Moreover, the activity of POD was estimated using peroxidase activity assay kit (Elabsience, United States, Product No.E-BC-K227-S) in accordance to set protocol of manufacturer.

#### 2.4.2. Grain carbohydrate content

Grain carbohydrate content (GCC) was measured using the Anthrone method followed by Madar et al. (2017). For this purpose

0.5 g powdered seed sample was mixed with diluted sulphuric acid in a test tube followed by the addition of anthrone reagent. The solution was boiled and then cooled to record the absorbance at 620 nm for taking the readings for total carbohydrate contents.

#### 2.5. Measurement of agronomical traits

Agronomical traits for instance plant height (PH) and pod length (PL) were calculated using scale while leaf area index (LAI) was calculated using plant canopy analyzer. Moreover branches per plant (BPP) and pods per plant (PPP) were calculated from randomly selected plants and averaged for analysis. All pods were manually threshed to record hundred seed weight (HSW) using electronic weighing balance.

**Table 2**

Overall significance of correlation coefficients among physiological traits, biochemical parameters and agronomic traits at drought and salinity stresses due to humic acid applications among mungbean genotypes.

|                  |         |         |          |          |          |          |          |          |          |          |          |          |          |          |          |
|------------------|---------|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| <b>Total Chl</b> | 0.97*** | 0.95*** | -0.91*** | 0.92***  | 0.91***  | -0.97*** | 0.91***  | 0.97***  | 0.97***  | 0.94***  | 0.97***  | 0.99***  | 0.97***  | 0.92***  | 0.91***  |
| <b>Pn</b>        |         | 0.93*** | -0.88*** | 0.94***  | 0.90***  | -0.93*** | 0.92***  | 0.95***  | 0.93***  | 0.94***  | 0.96***  | 0.96***  | 0.98***  | 0.92***  | 0.92***  |
| <b>Tr</b>        |         |         | -0.91*** | 0.95***  | 0.90***  | -0.92*** | 0.89***  | 0.96***  | 0.94***  | 0.96***  | 0.91***  | 0.94***  | 0.93***  | 0.94***  | 0.93***  |
| <b>MD</b>        |         |         |          | -0.88*** | -0.92*** | 0.92***  | -0.95*** | -0.89*** | -0.89*** | -0.90*** | -0.90*** | -0.94*** | -0.90*** | -0.92*** | -0.95*** |
| <b>Gs</b>        |         |         |          |          | 0.91***  | -0.88*** | 0.91***  | 0.91***  | 0.90***  | 0.97***  | 0.86***  | 0.90***  | 0.91***  | 0.94***  | 0.95***  |
| <b>SOD</b>       |         |         |          |          |          | -0.94*** | 0.95***  | 0.89***  | 0.84***  | 0.94***  | 0.89***  | 0.92***  | 0.93***  | 0.93***  | 0.93***  |
| <b>Proline</b>   |         |         |          |          |          |          | -0.91*** | -0.93*** | -0.93*** | -0.92*** | -0.94*** | -0.96*** | -0.95*** | -0.92*** | -0.92*** |
| <b>CAT</b>       |         |         |          |          |          |          |          | 0.90***  | 0.85***  | 0.92***  | 0.91***  | 0.94***  | 0.93***  | 0.93***  | 0.94***  |
| <b>POD</b>       |         |         |          |          |          |          |          |          | 0.94***  | 0.95***  | 0.94***  | 0.97***  | 0.96***  | 0.91***  | 0.89***  |
| <b>PH</b>        |         |         |          |          |          |          |          |          |          | 0.89***  | 0.94***  | 0.95***  | 0.92***  | 0.89***  | 0.89***  |
| <b>LAI</b>       |         |         |          |          |          |          |          |          |          |          | 0.90***  | 0.93***  | 0.94***  | 0.97***  | 0.96***  |
| <b>BPP</b>       |         |         |          |          |          |          |          |          |          |          |          | 0.98***  | 0.98***  | 0.90***  | 0.89***  |
| <b>PPP</b>       |         |         |          |          |          |          |          |          |          |          |          |          | 0.97***  | 0.92***  | 0.92***  |
| <b>HSW</b>       |         |         |          |          |          |          |          |          |          |          |          |          |          | 0.94***  | 0.92***  |
| <b>PL</b>        |         |         |          |          |          |          |          |          |          |          |          |          |          |          | 0.98***  |
| <b>GCC</b>       |         |         |          |          |          |          |          |          |          |          |          |          |          |          |          |

Pn, photosynthesis rate; Gs, stomatal conductance; Tr, transpiration rate; MD, membrane damage; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; GCC, grain carbohydrate content; PH, plant height; BPP, branches plant<sup>-1</sup>; PL, pod length; LAI, leaf area index; PPP, pods plant<sup>-1</sup>; HSW, hundred seed weight.

\*Significant at  $P \leq 0.05$ .

\*\*Significant at  $P \leq 0.01$ .

\*\*\*Significant at  $P \leq 0.001$ .

## 2.6. Gene expression studies

The RNA was extracted from selected mungbean genotypes using Qiagen RNeasy kit (Qiagen, United States) using the protocol followed by Li et al. (2018). Afterward following the protocol of Li et al. (2018), cDNA library was constructed and for this purpose total 2 µg of RNA was used as per manufacturer instructions. Likewise, in accordance to manufacturer instructions SYBR Green 1 master kit was used for qRT-PCR analysis. Furthermore, the expression of genes was normalized using Actin-expressing gene (*Vrad03g00210*). The list of primers used in the study is indicated in Table 1.

## 2.7. Statistical analysis

Statistix 8.1 (McGraw-Hill 2008) software was used for estimating the analysis of variance (ANOVA) at a 5% probability level while RStudio version 1.3.959 (RStudio Team 2020) was used for correlation, principal component analysis (PCA) and heatmap analysis.

## 3. Results

### 3.1. Physiological traits

All physiological traits such as total chl, Pn, Gs, Tr and MD illustrated significant ( $p \leq 0.05$ ) variation in all genotypes in the presence of humic acid (HA) under both individual and integrated applications of drought and salinity stresses as compared to their respective controls (Fig. 1). During both drought and salinity stresses, total chl, Pn, Gs and Tr showed significant ( $p \leq 0.05$ ) decrease in all genotypes at HA<sub>0</sub> compared to HA, however this reduction was more prominent at integrated application of both stresses (Fig. 1a–d). Among genotypes, NM-121-25 (V1) depicted the least amount of these aforementioned traits while PRI-Mung-2018 (V4) depicted the highest. Conversely, HA<sub>0</sub> recorded dramatic increase in MD as compared to HA during both types of stresses, while this increase was more robust under integrated application of drought and salinity stresses (Fig. 1e). During both drought and salinity stresses, NM-121-25 (V1) illustrated the highest while PRI-Mung-2018 (V4) illustrated the lowest MD (Fig. 1e). In general all mungbean cultivars manifested increase in total chl (1.25–1.75 gkg<sup>-1</sup>), Pn (25–40 µmm<sup>-2</sup>S<sup>-1</sup>), Gs (750–850 mmm<sup>-2</sup>S<sup>-1</sup>) and Tr

(13–15.5 mmm<sup>-2</sup>S<sup>-1</sup>) under drought and salinity stresses due to humic acid application (HA) as compared to no humic acid application (HA<sub>0</sub>). Contrarily, the highest percentage of MD (15–38%) was recorded in all mungbean genotypes in the absence of humic acid (HA<sub>0</sub>) under both stresses as compared to the presence of humic acid (HA).

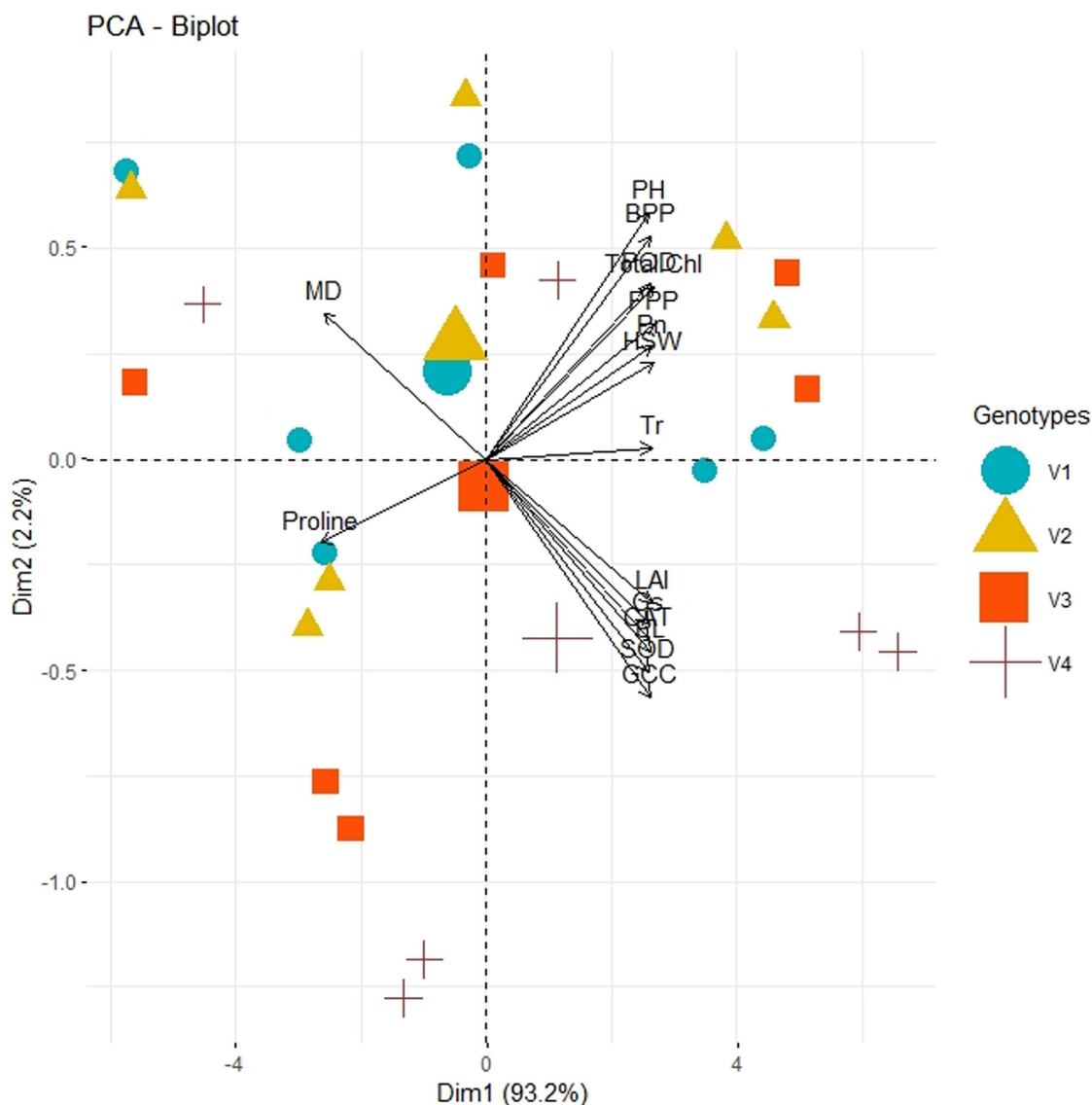
### 3.2. Biochemical parameters

All biochemical parameters including proline, antioxidant enzymes (SOD, CAT, POD) and GCC illustrated significant ( $p \leq 0.05$ ) variations during both drought and salinity stresses under both absence (HA<sub>0</sub>) and presence of humic acid (HA) (Fig. 2). Proline content illustrated significant increase (32–40 µg g<sup>-1</sup>FW) in all mungbean genotypes during drought salinity stresses at HA<sub>0</sub> compared to HA, however this increase was more dramatic (40 µg g<sup>-1</sup>FW) for integrated application of drought and salinity stresses (Fig. 2a). Among genotypes, NM-121-25 (V1) recorded the highest (40 µg g<sup>-1</sup>FW) proline content while PRI-Mung-2018 (V4) recorded the lowest (32 µg g<sup>-1</sup>FW). Besides, all antioxidant enzymes such as SOD, CAT and POD illustrated consistent increase in their activities among all genotypes at HA as compared to HA<sub>0</sub> under both drought and salinity stresses (Fig. 2b–e). Moreover, all enzymes depicted maximum activities in terms of enzyme units (SOD; 40, POD; 0.6, CAT; 16) due to HA in genotype PRI-Mung-2018 (V4) while least (SOD; 30, POD; 0.4, CAT; 10) in genotype NM-121-25 (V1) during both abiotic stresses. On the other hand, GCC illustrated significant ( $p \leq 0.05$ ) increase (55–60%) in all genotypes at HA compared to HA<sub>0</sub> during both drought and salinity stresses with maximum increase in PRI-Mung-2018 (V4) followed by DM-3 (V3), Chakwal M-6 (V2) and NM-121-25 (V1) as shown in Fig. 2f.

### 3.3. Agronomic traits

Application of humic acid (HA) significantly ( $p \leq 0.05$ ) improved the mean values of all agronomic traits such as PH, BPP, PL, LAI, PPP and HSW during both drought and salinity stresses in all mungbean cultivars (Fig. 3a–f). Both drought and salinity showed more negative impact on all these agronomic traits during no application of humic acid (HA<sub>0</sub>), however integrated application of drought and salinity exhibited more drastic impact. Besides, HA





**Fig. 4.** PCA scattered plot graph representing physiological, biochemical and agronomical parameters clustered on the basis of similarity and dissimilarity due to mungbean cultivars.

application made maximum increase in the mean values of agronomic traits (PH; 45–48 cm, BPP; 20–30, PL; 15–18 cm, LAI; 2–2.8, PPP; 20–25, HSW; 4–6 g) with the highest increase in PRI-Mung-2018 (V4) followed by DM-3 (V3), Chakwal M-6 (V2) and NM-121-25 (V1) during both drought and salinity stresses.

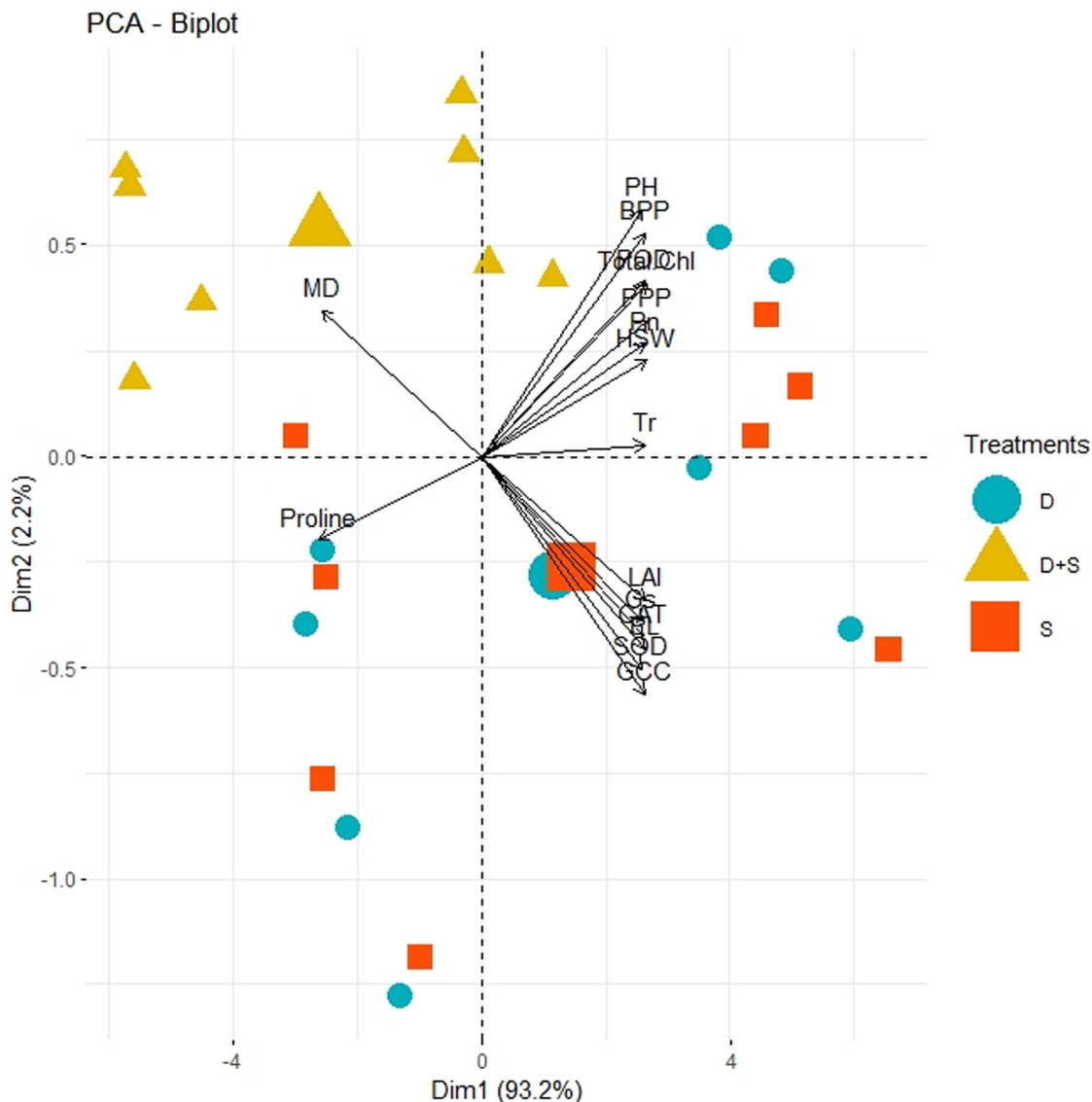
### 3.4. Overall correlation analysis

All traits illustrated significant correlation with each other in all genotypes under drought and salinity stresses due to presence (HA) and absence (HA<sub>0</sub>) of humic acid. During paired association analysis, all traits depicted positive association, except MD and proline that depicted negative association (Table 2). Besides, PCA for different genotypes of mungbean revealed prominent difference in the extent of association of all traits under study (Fig. 4), however genotype PRI-Mung-2018 exhibited maximum divergence from proximity. On the other hand, all traits showed approximately same degree of association both under drought and salinity stresses, however all traits showed maximum divergence under integrated application of drought and salinity stresses

(Fig. 5). Likewise, application of humic acid (HA) made differential impact on the association of all traits as compared to no humic acid application (HA<sub>0</sub>) as indicated in Fig. 6.

### 3.5. Multi-factor and heatmap analysis

Multi factor analysis map indicated prominent difference in the performance of all genotypes under drought and salinity stresses during HA compared to HA<sub>0</sub> as shown in Fig. 7 in the form of different circles. Besides, both drought and salinity illustrated approximately same effect on the performance of all mungbean genotypes, however their integrated application showed differential impact on genotypes as indicated by separate circle on multi factor analysis map (Fig. 7). On the other hand, heatmap analysis revealed two major clusters in context of trait expression under drought and salinity stresses due to presence (HA) and absence (HA<sub>0</sub>) of humic acid (Fig. 8). The integrated application of drought and salinity stresses affected the expression all of traits except MD and proline more negatively under no humic acid (HA<sub>0</sub>) application compared to humic acid application (HA). Overall, HA



**Fig. 5.** PCA scattered plot graph representing physiological, biochemical and agronomical parameters clustered on the basis of similarity and dissimilarity due to individual and integrated drought and salinity treatments.

application improved the expression of all traits in all mungbean genotypes compared to  $HA_0$ , however this improvement was more prominent in PRI-Mung-2018 (V4) followed by DM-3 (V3), Chakwal M-6 (V2) and NM-121-25 (V1).

### 3.6. Gene expression analysis

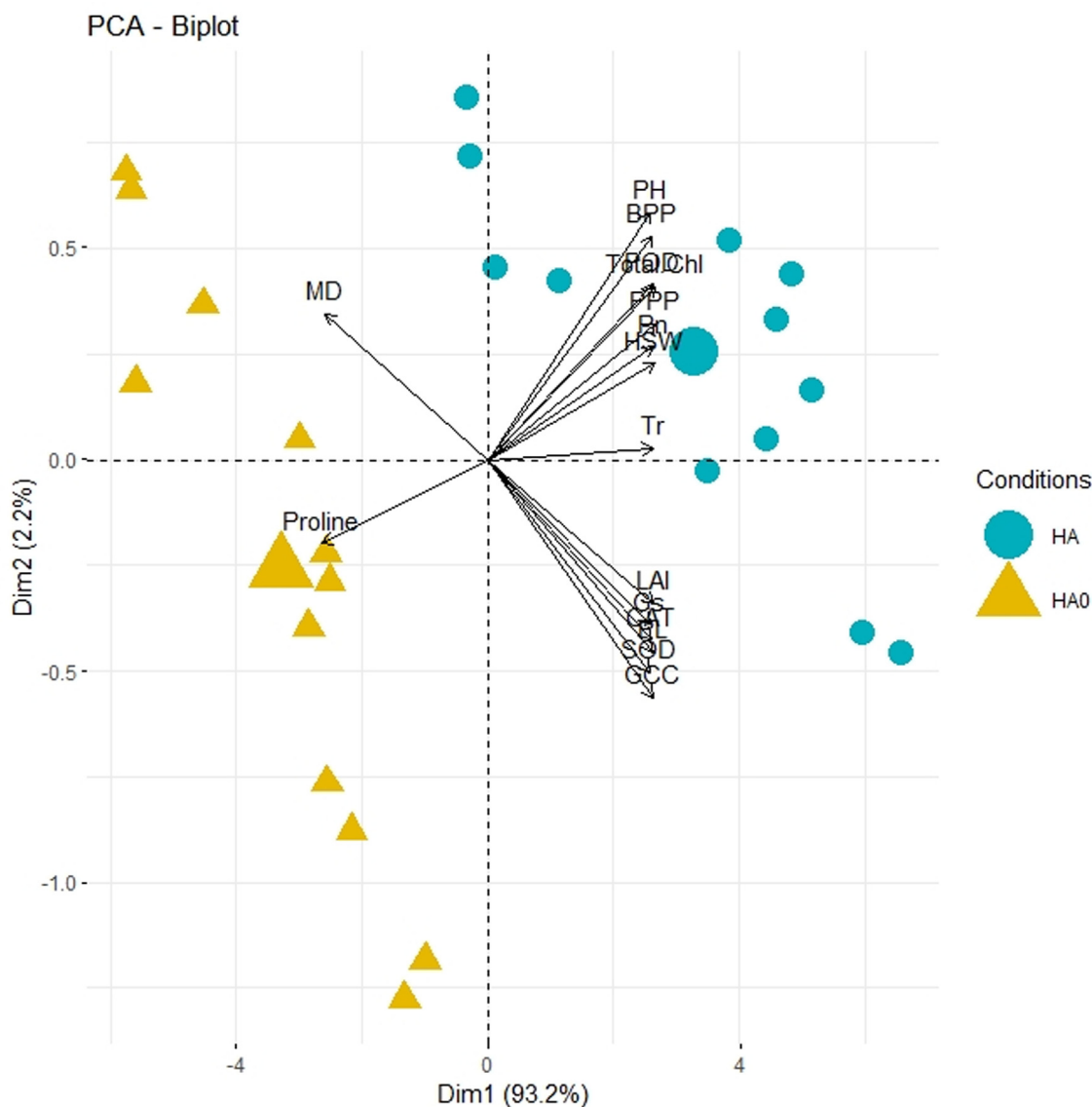
The expression of all genes under study was significantly altered under both drought and salinity stresses. Under no humic acid application ( $HA_0$ ), the relative expression of both drought and salinity related genes showed significant difference in all four mungbean genotypes (Fig. 9). Besides, under drought stress all genes *VrHsfA6a*, *VrDREB2a*, *VrZBP17* showed up-regulation in all genotypes, however they revealed comparatively high expression in mungbean genotype NM-121-25 (V1) followed by Chakwal M-6 (V2), DM-3 (V3) and PRI-Mung-2018 (V4). Correspondingly, all salinity related genes *VrWRKY73*, *VrUBC1* and *VrNHX1* genes recorded relatively low expression in all genotypes under humic acid application (HA) as compared to control ( $HA_0$ ) with genotype NM-121-25 (V1) the highest while PRI-Mung-2018 (V4) the low-

est. Besides, under integrated application of drought and salinity both drought and salinity related genes showed up-regulation, whereas their expression was maximum in NM-121-25 (V1) while minimum in PRI-Mung-2018 (V4). Contrarily, in presence of humic acid application (HA) expression of both drought and salinity related genes were down-regulated as compared to no application of humic acid ( $HA_0$ ). This trend was more dramatic in mungbean genotype PRI-Mung-2018 (V4) followed by DM-3 (V3), Chakwal M-6 (V2) and NM-121-25 (V1).

### 4. Discussion

Current study explicated the role of humic acid application in alleviating the negative impacts of drought and salinity stresses at physiological, biochemical and agronomical levels in different genotypes of mungbean. Humic acid significantly countered the deleterious effects of drought and salinity and improved mungbean agronomic production by optimizing various physiological and genetic responses. Abiotic stresses impair crop productivity by hampering the physiological processes including Pn and Gs

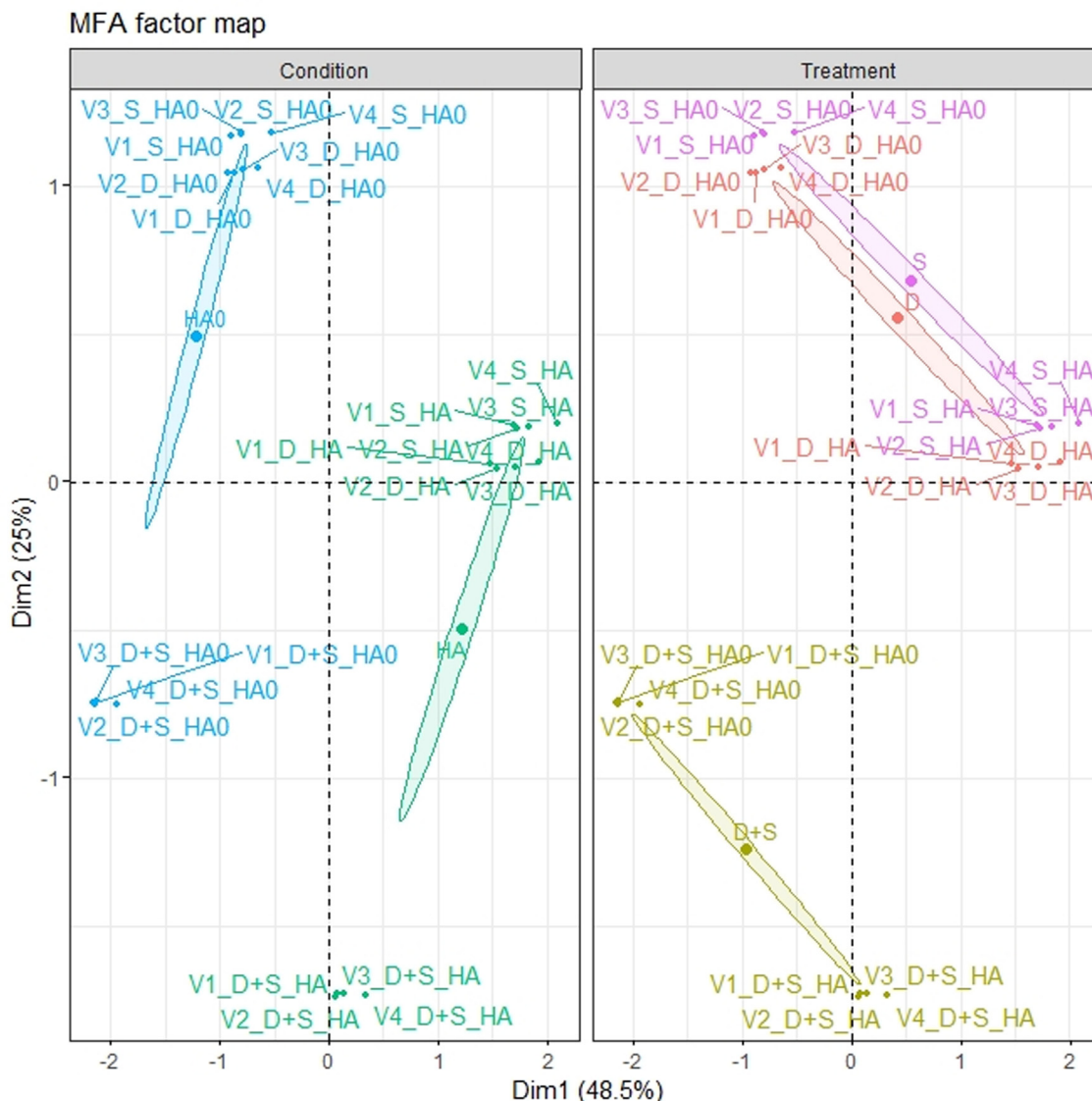




**Fig. 6.** PCA scattered plot graph representing physiological, biochemical and agronomical parameters clustered on the basis of similarity and dissimilarity due to presence (HA) and absence (HA0) of humic acid.

due to damage of cell membrane and photosystems (Alghabari et al., 2021). Likewise, drought negatively affects Pn due to reduction in leaf area and damage of photosynthetic machinery including total chlorophyll content (Bangar et al., 2019). Besides, disruption in osmotic balance of cells due to drought and salinity stresses perturbs the fluidity of cell membrane leading towards more electrolyte leakage owe to cell membrane damage. These all factors collectively disturbs the physiological coherence of plants causing reduction in Pn, Gs and Tr as reviewed by HanumanthaRao et al. (2016) and Singh et al. (2021) in mungbean. Current study correspondingly endorsed their findings by observing consistent decrease in Pn, Gs and Tr in all mungbean genotypes due to reduction in total chlorophyll content and increasing MD (Fig. 1). On the other hand, plant biostimulants such as humic acid are frequently being integrated in production systems for triggering physiological processes with objective to boost productivity (Yakhin et al., 2017). In fact humic acid acts as soil conditioner with tendency to improve soil structure and chemistry that results in more efficient water and nutrient uptake even during the conditions of abiotic stresses (Alghabari, 2020). This leads to more

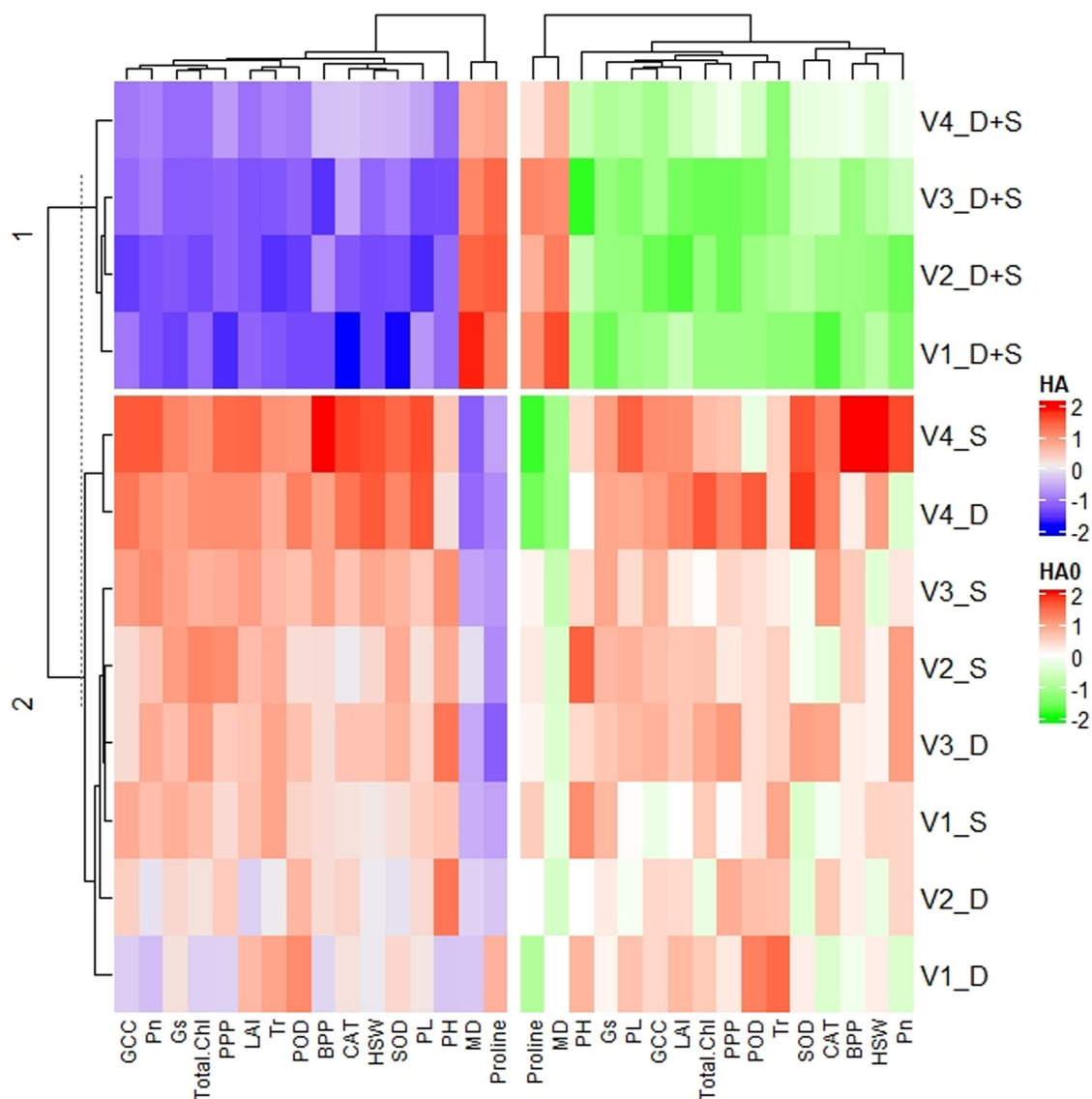
nutrient assimilation including Mg which improves total chlorophyll content, LAI leading toward high Pn and productivity as reported in present study. Moreover, the application of humic acid improves Pn under water stress due to enhanced Gs (Lotfi et al., 2018). Correspondingly in current study, humic acid increased total chl, Pn, Gs and Tr in all mungbean genotypes that rendered significant effect on crop agronomic productivity (Figs. 1 and 3). Complementary to the findings of the present study, Bangar et al. (2019) also reported statistically significant increase in physiological parameters of mungbean such as Pn, Gs and Tr due to humic acid application under stress. Besides, both drought and salinity stresses trigger the production of ROS in plants due to decline in the activities of antioxidant enzymes such as SOD, CAT and POD (Shah et al. 2018) as confirmed by present study (Fig. 2). Application of humic acid under saline conditions reduces the generation of ROS in common bean (*Phaseolus vulgaris* L) by triggering the activities of antioxidant enzymes SOD, CAT and POD resulting in least MD and less electrolyte leakage (Aydin et al., 2012). Similarly, Abbas et al. (2022) observed improvement in wheat tolerance against drought and salinity under humic acid application due to



**Fig. 7.** Graphical description of multiple factor analysis indicating the effects of humic acid conditions (HA0, absence; HA, presence), drought and salinity treatments (D, drought; S, salinity; D + S, drought + salinity) on mungbean genotypes.

initiation of numerous physiological and biochemical activities, like enhanced Gs, and activation of the antioxidant enzymes such as CAT, POD and SOD to mitigate the hazards of ROS. In complement to their findings, current study reported consistent increase in the activities of antioxidant enzymes in all mungbean genotypes under both drought and salinity stresses due to application of humic acids (Fig. 2). These antioxidant enzymes play vital part in nullifying the effect of ROS generated in plants during drought and salinity stresses (García et al., 2012). This could be attributed to the ability of humic acid to differentially regulate the ATPases residing in tonoplast and cell membrane that counter the hazardous effect of ROS and hence impart more membrane stability and less damage (Zandonadi et al., 2016; Hasanuzzaman et al., 2021). To cope with oxidative stress induced by drought or salinity, plants use natural homeostatic defense strategy such as readjusting osmotic balance by triggering the production of osmolytes such as proline (Shah et al., 2017) as authenticated by current study (Fig. 2). Contrary to the findings of Aydin et al. (2012) in common bean, current study reported decrease in proline content in all mungbean genotypes under drought and salinity stresses due to

application of humic acid (Fig. 2). This can be attributed to the tendency of humic substances as stress reliever as reviewed by Shah et al. (2018). Moreover, humic acid has tendency to adhere the plant cell wall for absorption by roots from where it is transported via shoot to leaves where it affects plant physio-metabolic activities directly (Nardiet al., 2002; Asli and Neumann, 2010). Likewise, current study revealed strong correlation of all physiological and biochemical traits with agronomic yield of mungbean (Table 2). Humic acid improves PH, BPP, LAI and total chl to harvest more sunlight leading towards higher Gs and Pn that result in accumulation of more photosynthates, hence yield more PPP, PL and grain (Alghabari, 2020). In consistent with these findings, current study proved this linkage by elucidating strong correlation among physiological (total Chl, Pn, Gs and Tr) biochemical (antioxidant enzymes) and agronomical traits (PH, BPP, LAI, PL, PPP and HSW) in all mungbean genotypes (Table 2, Figs. 4–6). Complementary to our findings Waqas et al. (2014) reported noteworthy increase in mungbean yield under the application of humic acid as compared to respective control under the conditions of stress. Furthermore, Alghabari (2020) noticed significant impact of HA



**Fig. 8.** Cluster dendrogram heatmap indicating the responses of physiological, biochemical and agronomical traits to mungbean genotypes at individual and integrated treatments of drought and salinity stresses under absence (HA0) and presence (HA) of humic acid.

on the grain carbohydrate content of mungbean. Humic acid has tendency to stimulate nutrients uptake by improving the physiochemical property of soil even during salinity and drought stresses that leads to more accumulation of photosynthates such as carbohydrates due to speedy  $\text{CO}_2$  fixation owe to increased stomatal conductance (Shah et al., 2018). Moreover, this connection of physiological and biochemical events in boosting agronomical yield has been reported in various studies (Waqas et al., 2014; Madar et al., 2017; Ozlem et al., 2017; Alghabari, 2020; Bangar et al., 2019). Besides, all mungbean genotypes (PRI-Mung-2018 (V4), DM-3 (V3), Chakwal M-6 (V2) and NM-121-25 (V1)) showed differential responses to individual as well as integrated application of drought salinity stress due to humic acid application (Figs. 7 and 8) that can be attributed to their varying tendency of susceptibility to biostimulants as reviewed by Shah et al. (2018).

Besides, plants like every living being are equipped with ability to cope with stress by activating their homeostatic machinery based upon the regulation of genetic determinants (Shah et al., 2017). Likewise, other stresses plants also handle the abiotic stresses such as drought and salinity by regulating their genetic

mechanisms (Srivastava et al., 2018). Labbo et al. (2018) explained the high expression of drought tolerant genes *VrDREB2A*, *VrbZIP17* in mungbean genotypes under drought stress is a consequence of plant drought countering response that triggers the accumulation of osmolytes preventing the plants against drastic impacts of stress. Meanwhile high expression of gene *VrHsfA6a* in mungbean under stress also explicates the genetic regulation in plant render by drought stress (Liu et al., 2016). Correspondingly, current study recorded the up-regulation of all these aforementioned genes in all mungbean genotypes that was an indicator of plant genetic response to stress (Fig. 9). On the other hand, varying expression of salinity related genes such as *VrWRKY73*, *VrUBC1* and *VrNHX1* in mungbean genotypes is also an indicator of their varying tendency of osmotic adjustment to handle the salinity induced stress as explicated by Srivastava et al. (2018). Current study has authenticated their outcomes by reporting the differential expression of salinity related genes among mungbean genotypes (Fig. 9). Humic acid is a plant tonic that not only boosts plant productivity instead also acts as stress reliever (Shah et al. 2018). The down regulation of salinity and stress tolerant genes in presence of humic acid



**Fig. 9.** Relative expression of salinity (*VrWRKY73*, *VrUBC1* and *VrNHX1*) and drought (*VrDREB2A*, *VrbZIP17* and *VrHsfA6a*) related genes in different mungbean genotypes under absence (HA0) and presence of humic acid (HA) during individual and integrated application of drought stress. V1, NM-121-25; V2, Chakwal M-6; V3, DM-3; V4, PRI-mung-2018.

during both individual and integrated applications of drought and salinity stresses has proved the effect of humic acid on gene regulation as reviewed by [Shah et al. 2018](#). In this perspective mungbean genotype PRI-Mung-2018 depicted noteworthy performance.

## 5. Conclusion

Overall, current research proved mungbean cultivar, PRI-Mung-2018 well adapted and responsive in field conditions of under both



individual and integrated treatments of drought and salinity stresses due to humic acid application. Therefore, it is recommended to expand the cultivation of this cultivar with foliar application of humic acid in prevailing soil conditions of Saudi Arabia where drought and salinity are potential challenges. Moreover, humic acid is an important biostimulant to strengthen the connection between physiological, biochemical, molecular and agronomic attributes of mungbean for boosting its productivity in stressed soil of Saudi Arabia.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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