Terminal Drought-priming Improves the Drought Tolerance in Desi and Kabuli Chickpea

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Abstract

Drought stress is one of the principal factors responsible for low productivity of chickpea. In this study, the influence of terminal drought-priming in improving the drought tolerance in desi and kabuli chickpea types was evaluated. During first season, both chickpea types were grown in soil filled pots under well-watered conditions (75% water holding capacity). At flowering, half of the pots were kept under well-watered conditions while rest half were maintained under drought (50% water holding capacity). Terminal drought stress significantly affected the seed composition of both chickpea types as indicated by increase in total proteins (10%), zinc (9.5%), potassium (3.2–0.9%), calcium (2.5–1.3%), and total soluble phenolics (4–57%) compared with the plants raised under well-watered conditions. During second season, chickpea seeds collected from well-watered and droughted source were planted in soil filled pots under drought (50% water holding capacity) and well-watered conditions (75% water holding capacity). Drought stress suppressed the stand establishment, seedling growth, carbon assimilation, PSII efficiency, total chlorophyll contents, α-amylose activity, sugar metabolism, and trehalose contents of both chickpea types. Chickpea types also differed in their response to drought; kabuli chickpea type was more affected by drought than the desi type. The desi chickpea type had better stand establishment and growth than the kabuli chickpea type. However, terminal drought-priming improved the performance of both chickpea types under drought; nonetheless the improvement was more pronounced in desi chickpea types. Terminal drought-priming improved the germination metabolism and accumulation of free proline, total phenolics and trehalose contents, which assisted desi chickpea to perform well under drought. In conclusion, changes in seed composition induced by drought-priming improved drought tolerance in chickpea are owed to better germination, carbon assimilation and accumulation of free proline, total soluble phenolics and trehalose.

Keywords: Drought priming; Proline; Phenolics; PSII; Carbon assimilation

Introduction

Drought is one of the major factors, which impede the growth and development of chickpea. Drought stress disrupts the assimilation of carbon and other minerals (Farooq et al., 2009; Jaleel, et al., 2009), which causes decrease in plant growth and productivity (Farooq et al., 2017a). Drought effects the photosynthetic apparatus due to stomatal control of CO₂ supply, reduction in carbon cycle (Awasthi et al., 2014), destruction of thylakoid membranes and disturbance of chlorophyll pigment formation (Tas and Tas, 2007; Farooq et al., 2009). Decrease in moisture availability causes decrease in plant growth on the account of decrease in photosynthetic capacity and area (Wahid and Rasul, 2005; Chaves et al., 2011). Under drought, the abscission and senescence increases, and formation of new leaves is decreased (Karamanos, 1980), therefore the total leaf area is decreased significantly (Farooq et al., 2017a).

In leaves decline in influx of carbon (Chaves, 1991; Awasthi et al., 2014) and the chlorophyll contents under water deficit conditions may cause chloroplast damage due to oxidative stress (Farooq et al., 2017a) through dilation of thylakoid membrane and destabilization of protein pigment complex (Prasad and Saradhi, 2004). These conditions led to the increase in production of reactive oxygen species (ROS; such as superoxide, O₂⁻; hydroxyl radicle OH; and hydrogen peroxide) (McCord, 2000; Ruelland et al., 2009). These ROS cause cellular injury to DNA, nucleic acids, lipids, and proteins and cause cell death (Foyer, 2005).

Terminal drought stress significantly reduced the grain yield in chickpea due to abortion of flower and pod, reduced seed size, decreased pod production (Davies et al., 1999;
Fang et al., 2010) and impaired pollen viability and stigma/style functionality (Fang et al., 2010). Drought stress reduced the carbon cycle and disrupts the electron transport, by decreasing the stomatal supply of CO$_2$ (Allen and Ort, 2001; Awasthi et al., 2014), nutrient absorption and their utilization due to reduced transpiration (Faroq et al., 2009) and increased apoplastic abscisic acid concentration, which cause closing of stomata (Liu et al., 2005). Reduction in influx of carbon into the leaves under the moisture stress is the early response of plants to close the stomata to stop the water loss (Awasthi et al., 2014), decrease in internal CO$_2$ supply cause reduction in photosynthesis owing to decline in ATP synthase activity and Rubisco (ribulose-1, 5-bisphosphate carboxylase) (Zlatev and Lidon, 2012; Farooq et al., 2017a).

Priming (pre-exposure of plants to stress), permits plants to become more tolerant to stress occurring later (abiotic or biotic) (Bruce et al., 2007). The pre-exposure to drought enhances the elasticity of plants to cope with re-occurring of same stress (Ding et al., 2012). During the developmental cascades, plants are able to conserve the episode of previous stress to a subsequent stress (Wang et al., 2014). The stress-primed plants facing an early drought stress event had better photo-protection and a higher biomass than non-primed in second drought episode (Walter et al., 2011). In another study, the drought primed plants exhibited improved photosynthesis rate, leaf water status, ascorbate peroxidase and lower membranes damage than non-primed plants under later drought episodes (Wang et al., 2014). The exposure of plants to one stress improves its ability to tolerate the subsequent stresses in the next generation (Molinier et al., 2006; Cuk et al., 2010) by retaining the trans-generational stress memory (Walter et al., 2013). These stresses involve modifications in proteins, compatible solutes (Joyce et al., 2003) and up-regulation of antioxidative enzymes in next generation (Cuk et al., 2010).

The accumulation of sugars specifically trehalose impart protection against abiotsic stresses to the plants by ceasing the protein denaturation, acting as free radicals scavenger, stabilizing the cell membranes (Benaroudj et al., 2001), protecting from oxidative damage and by maintaining the carbon assimilation (Farooq et al., 2017b). Trehalose prevents denaturing of biological membranes during dehydration (Elbein et al., 2003), through detoxifying ROS with binding at polar region of membranes of phosphate and proteins hydroxyl group (Benaroudj et al., 2001; Farooq et al., 2009).

In our recent study, Tabassum et al. (2017) reported better salt tolerance in wheat, during next generation, by drought-priming owing to the osmolytes accumulation and improved water relations. However, to the best of our knowledge, no information is available on the effect of terminal drought-priming on drought tolerance of chickpea. This study was, therefore, conducted to evaluate the influence of drought-priming on drought tolerance in desi and kabuli chickpea types.

Material and Methods

Plant Material

Seeds of desi chickpea (Bitall-2016) and kabuli chickpea (Noor-2013) were collected from the Pulses Research Institute, Faisalabad, Pakistan.

Year 1

Drought-Priming

Seeds of both chickpea types were planted six in each pot in soil filled pots (10 kg) under natural conditions with adequate water supply maintained at 75% water holding capacity. Upon the uniformity of seedling emergence, seedlings were thinned to maintain three plants in each pot. At flowering, half of pots were kept under well-watered conditions while rest half were maintained under drought (50% water holding capacity). At harvest maturity, plants were harvested, sun-dried for three days, and were threshed to separate the seeds.

Seed Composition Analysis

The chickpea seeds, from both drought stressed and well-watered sources, were milled to make powder. Total soluble proteins were determined following Bradford method (Bradford, 1976). For the determination of total soluble phenolics, the flour was overnight soaked in 80% acetone then Folin-Ciocalteu reagent and Na$_2$CO$_3$ solution were added. Total soluble phenolics were estimated as gallic acid equivalent (GAE) (Singleton and Rossi, 1965). Seed phosphorus (P), potassium (K), calcium (Ca$^{2+}$) and zinc (Zn$^{2+}$) were determined using Inductively coupled plasma (ICP) atomic absorption with a Perkin Elmer Optima 5300 DV optical emission spectrometer (OES; Shelton, CT, USA).

Year 2

Experimental Details

Seeds of both chickpea types, from droughted and well-watered sources, were sown (ten seeds per pot) in soil-filled plastic pots (5 kg) and were maintained at (i) 75% water holding capacity (well-watered), (ii) 50% water holding capacity (drought stress). After completion of emergence, three plants per pot were maintained through thinning. These pots were kept in a climate chamber having temperature (day/night) (18/15°C) with a photosynthetically active photon flux with a photoperiod (light/dark) of (16/8 h) having density (350 mM m$^{-2}$ s$^{-1}$). This study was performed under factorial arrangement having completely randomized design with six replications. Each replicate contained five pots having three numbers of plants per pot. The plants were harvested four weeks after the planting.
Stand Establishment

The experiment was visited daily, and number of seedlings emerged were counted daily until a constant count was achieved. Emergence index (EI) and the coefficient of uniformity of emergence (CUE) were estimated following Association of Official Seed Analysts (1983) and Bewley and Black (1985), respectively. The ratio of emerged seedlings to the number of seeds expressed in percentage was recorded as final emergence percentage (FEP).

α-Amylase Activity and Sugars

Two days after sowing five germinating seedlings were collected and crushed to determine α-amylase activity. The sap was mixed with phosphate buffer having pH 0.7 and was incubated for 24 h at 4°C. From the supernatant, α-amylase activity was determined by dinitrosalicylic acid (DNS) method (Bernfeld, 1955) modified by Lee and Kim (2000). For the determination of total soluble sugars, the ground seed samples were mixed with distilled water and were incubated at 25°C for 24 h (Lee and Kim, 2000). The filter paper (Whatman No. 42) was used to filter the mixture and the filtrate was used for the determination of total soluble sugars using the phenol-sulfuric acid method (DuBois et al., 1956). Taking glucose as standard, the reducing sugars were estimated from the filtrate used for total sugars by using the method of (Miller, 1959). From the same filtrate, the sucrose contents were determined following Stitt et al. (1989), for the determination of trehalose contents, ground seed samples were centrifuged at 10,000× g for 10 min. The trehalose was determined by estimating the glucose produced by hydrolysis of trehalose using a glucose oxidase-peroxidase kit (Spanireact) given by Čižmárilk et al. (2004), by incubating the supernatant with pH adjusted at 7.0, in a boiling water bath for 60 min.

Leaf Photosynthesis, PSII Efficiency and Total Chlorophyll Contents

Leaf net CO₂ assimilation rate was estimated with a portable infrared gas analyzer based photosynthesis system (LI-6400; LiCor, Inc., NE, USA) between 9:00 and 11:00 one day before final harvest. Using Handy PEA (Plant Efficiency Analyzer, Hansatech, Norfolk, UK) with an excitation light energy of 3000 umol m⁻² s⁻¹, the maximum efficiency of PSII in chickpea leaves was determined. Following the method described by Arnon (1949) the total leaf chlorophyll contents were determined.

Lipid Peroxidation, Total Soluble Phenolics and Free Leaf Proline

Malondialdehyde (MDA) contents were estimated as an index of lipid peroxidation. Leaf samples (1 g) were homogenized in 10 mL of 0.1% trichloroacetic acid solution. The MDA contents were estimated using the method of Heath and Packer (1968). Leaf total soluble phenolics were estimated as described above for seed composition analysis. To determine the leaf free proline, leaf samples were homogenized in 10 mL of aqueous sulphosalicylic acid (w/v) and then filtered. The filtrate (2 mL) was mixed with 2 mL each of ninhydrin and glacial acid and incubated for 1 h at 100°C in a water bath. After vortexing for 20 sec, the reaction was terminated in an ice bath and 4 mL toluene, and chromatophore containing proline was aspirated, added to a test tube. The leaf free proline was determined following Bates et al. (1973).

Plant Growth

Leaves were separated from the harvested plants to record the leaf area by using leaf area meter (DT Area Meter, Model MK2; Delta-T Devices, Cambridge, UK). The same leaves were then dried in an electric oven at 70±2°C for 96 h, and specific leaf area was recorded as the ratio of leaf area to leaf dry weight. Dry weight of all above ground plant material was recorded as seedling dry weight.

Leaf Mineral Analysis

For the determination of leaf minerals dried leaves were ground to pass through a 1-mm screen of an Udy Cyclone Mill (Udy Corp., Ft. Collin, CO). Total leaf nitrogen (N) was estimated by combustion analysis (CHN-1000 analyzer; LECO Corp., St. Joseph, MO). Leaf P, K and Ca⁺² were estimated as described above for seed composition analysis.

Statistical Analysis

The experiment data were analysed statistically by analysis of variance technique (statistical software Co-Stat; CoHort, Berkeley, CA, USA). Least significant difference (LSD) test was used for the mean separation at 5% probability level (Steel et al., 1997).

Results

Seed Composition

Terminal drought-priming stress significantly affected the seed composition of both chickpea types (desi and kabuli chickpea). Terminal drought stress increased total proteins (10%), Zn (9.5%), K (3.2 and 0.9%), Ca⁺² (2.5 and 1.3%), and total soluble phenolics (41 and 57%) in desi and kabuli chickpea types, respectively, compared with well-watered (Table 1).

Stand Establishment and Growth

Drought stress suppressed the stand establishment and seedling growth of chickpea. However, both tested chickpea types significantly differed for response to drought stress from both seed sources (drought-priming and well-watered) (Table 2).
There was no difference between drought-priming and well-watered seed sources for CUE, emergence index, and final emergence percentage in both chickpea types under well-watered conditions. Under well-watered conditions, although there was no difference in seedling dry weight of desi chickpea type from either seed source, however, drought-priming caused reduction in seedling dry weight of kabuli chickpea type and SLA in both chickpea types (Table 2). Under drought stress, better CUE, emergence index, final emergence percentage, seedling dry weight and SLA were noted from drought-primed seeds than the well-watered seed source in both chickpea types (Table 2).

**α-Amylase Activity and Sugars Metabolism**

Both tested chickpea types significantly differed for α-amylase activity, total soluble sugars, sucrose, reducing sugars and trehalose contents, under well-watered and drought conditions irrespective of the seed source (Table 3). Under well-watered, desi chickpea had more α-amylase activity, reducing sugars, total soluble sugars, sucrose and trehalose contents than the kabuli chickpea type irrespective of seed source (Table 3).

In both tested chickpea types, drought stress significantly reduced the α-amylase activity, total soluble sugars, sucrose, reducing sugars and trehalose contents from both seed sources. However, drought-priming had more α-amylase activity, total soluble sugars, sucrose, reducing sugars and trehalose contents than the well-watered seed source under drought stress (Table 3).

**Leaf CO₂ Net Assimilation Rate, PSII Efficiency and Total Chlorophyll Contents**

The both tested chickpea types significantly differed for leaf CO₂ net assimilation rate, maximal PSII efficiency and total chlorophyll contents from both seed sources under both drought stress and well-watered condition (Table 4). Under well-watered conditions, drought-priming and well-watered seed source did not differ for leaf CO₂ net assimilation rate, maximal PSII efficiency and total chlorophyll contents. Drought caused significantly reduced the leaf CO₂ net assimilation rate, maximal PSII efficiency and total chlorophyll contents in both chickpea types from both seed source (Table 4).

Under drought stress, kabuli chickpea had lower leaf CO₂ net assimilation rate, maximal PSII efficiency and total chlorophyll contents, irrespective of seed source, than the desi chickpea (Table 4). However, under drought, drought-priming increased the lower leaf CO₂ net assimilation rate, maximal PSII efficiency and total chlorophyll contents in both chickpea types than the well-watered seed source (Table 4).

**Lipid Peroxidation, Free Leaf Proline and Total Soluble Phenolics**

Under well-watered conditions, except for total soluble phenolics, the both tested chickpea types significantly differed for leaf malondialdehyde contents, and free leaf proline irrespective of seed source (Table 5). However, drought stress caused significant increase in these parameters. Under drought stress, highest leaf malondialdehyde contents were noted in kabuli chickpea from well-watered seed source (Table 5). However, highest total soluble phenolics and free leaf proline contents were recorded in desi chickpea from drought-primed seeds, but, the lowest leaf malondialdehyde contents were noted in desi chickpea from drought-primed seeds (Table 5).
Table 3: Effect of drought-priming on α-amylase activity, total soluble sugars, reducing sugars, sucrose and trehalose of desi and kabuli chickpea under well-watered (WW) and drought stress (DS) conditions

<table>
<thead>
<tr>
<th>Chickpea types</th>
<th>Seed source</th>
<th>α-amylase activity (IU mg⁻¹ protein)</th>
<th>Total soluble sugars (mg g⁻¹)</th>
<th>Reducing sugars (mg g⁻¹)</th>
<th>Sucrose (mg g⁻¹)</th>
<th>Trehalose (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
</tr>
<tr>
<td>Desi</td>
<td>Well-watered</td>
<td>8.77a</td>
<td>5.54d</td>
<td>10.15a</td>
<td>6.45d</td>
<td>6.33a</td>
</tr>
<tr>
<td></td>
<td>Drought-priming</td>
<td>8.55a</td>
<td>6.75c</td>
<td>9.94a</td>
<td>7.15c</td>
<td>6.47a</td>
</tr>
<tr>
<td>Kabuli</td>
<td>Well-watered</td>
<td>7.67b</td>
<td>4.25e</td>
<td>8.75b</td>
<td>4.75f</td>
<td>5.84b</td>
</tr>
<tr>
<td></td>
<td>Drought-priming</td>
<td>7.53b</td>
<td>5.15f</td>
<td>8.51b</td>
<td>5.28e</td>
<td>5.81b</td>
</tr>
</tbody>
</table>

Any two means, for a parameter, not sharing a letter in common differ significantly at p ≤ 0.05

Table 4: Effect of drought-priming on leaf CO₂ net assimilation rate, PSII efficiency and total chlorophyll of desi and kabuli chickpea under well-watered (WW) and drought stress (DS) conditions

<table>
<thead>
<tr>
<th>Chickpea types</th>
<th>Seed source</th>
<th>Leaf CO₂ net assimilation rate (µmol s⁻¹ m⁻²)</th>
<th>PSII efficiency (Fv/Fm)</th>
<th>Total chlorophyll (mg g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
</tr>
<tr>
<td>Desi</td>
<td>Well-watered</td>
<td>10.68a</td>
<td>7.26d</td>
<td>0.71a</td>
</tr>
<tr>
<td></td>
<td>Drought-priming</td>
<td>10.72a</td>
<td>8.44c</td>
<td>0.69a</td>
</tr>
<tr>
<td>Kabuli</td>
<td>Well-watered</td>
<td>9.42b</td>
<td>6.96c</td>
<td>0.59b</td>
</tr>
<tr>
<td></td>
<td>Drought-priming</td>
<td>9.48b</td>
<td>7.34d</td>
<td>0.56b</td>
</tr>
</tbody>
</table>

Any two means, for a parameter, not sharing a letter in common differ significantly at p ≤ 0.05

Table 5: Effect of drought-priming on leaf malondialdehyde (MDA) contents, Total soluble phenolics and free leaf proline of desi and kabuli chickpea under well-watered (WW) and drought stress (DS) conditions

<table>
<thead>
<tr>
<th>Chickpea types</th>
<th>Seed source</th>
<th>Leaf MDA contents (µmol g⁻¹ FW)</th>
<th>Total soluble phenolics (µg g⁻¹ FW)</th>
<th>Free leaf proline (µmol g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
</tr>
<tr>
<td>Desi</td>
<td>Well-watered</td>
<td>11.25f</td>
<td>16.43c</td>
<td>15.54d</td>
</tr>
<tr>
<td></td>
<td>Drought-priming</td>
<td>11.34f</td>
<td>14.44d</td>
<td>15.66d</td>
</tr>
<tr>
<td>Kabuli</td>
<td>Well-watered</td>
<td>12.33c</td>
<td>19.76a</td>
<td>15.29a</td>
</tr>
<tr>
<td></td>
<td>Drought-priming</td>
<td>12.49e</td>
<td>17.32b</td>
<td>15.48d</td>
</tr>
</tbody>
</table>

Any two means, for a parameter, not sharing a letter in common differ significantly at p ≤ 0.05

Table 6: Effect of drought-priming on leaf nitrogen, leaf phosphorus, leaf potassium and leaf calcium of desi and kabuli chickpea under well-watered (WW) and drought stress (DS) conditions

<table>
<thead>
<tr>
<th>Chickpea types</th>
<th>Seed source</th>
<th>Leaf N (mg g⁻¹ DM)</th>
<th>Leaf P (mg g⁻¹ DM)</th>
<th>Leaf K (mg g⁻¹ DM)</th>
<th>Leaf Ca²⁺ (mg g⁻¹ DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
</tr>
<tr>
<td>Desi</td>
<td>Well-watered</td>
<td>3.45a</td>
<td>2.84d</td>
<td>1.88a</td>
<td>1.73b</td>
</tr>
<tr>
<td></td>
<td>Drought-priming</td>
<td>3.45b</td>
<td>2.90c</td>
<td>1.91a</td>
<td>1.75b</td>
</tr>
<tr>
<td>Kabuli</td>
<td>Well-watered</td>
<td>3.33b</td>
<td>2.56f</td>
<td>1.86a</td>
<td>1.76b</td>
</tr>
<tr>
<td></td>
<td>Drought-priming</td>
<td>3.31b</td>
<td>2.72e</td>
<td>1.87a</td>
<td>1.74b</td>
</tr>
</tbody>
</table>

Any two means, for a parameter, not sharing a letter in common differ significantly at p ≤ 0.05

Leaf Mineral Analysis

Except for Ca²⁺ under well-watered conditions and leaf P under both well-watered and drought conditions, the tested chickpea types significantly differed for leaf mineral contents irrespective of seed source (Table 6). Mineral contents of kabuli chickpea were more strongly affected than the desi chickpea from well-watered seed source (Table 6). Drought caused significantly decrease in leaf mineral contents in both chickpea types irrespective of seed source, however, the drought-induced decrease was less from drought priming seed.

Discussion

The progeny of drought stressed chickpea types (both desi & kabuli) performed better than well-watered under drought stress, due to decrease in total lipids and increase in total proteins, Zn, K, Ca²⁺ and total soluble phenolics (Table 1). These phenolic compounds help in scavenging the ROS acting as antioxidants (Weidner et al., 2009). Stress during grain development phase change the quality and composition of grains by accumulating certain secondary metabolites and this change help the plants to tolerate the reoccurrence of the same stress or any other (Cuk et al., 2010; Tabassum et al., 2017) through modifications in metabolome and proteome with enhanced expression of compatible solutes and proteins (Joyce et al., 2003) as was observed in this study (Tables 3 and 5). Plants maintain trans-generational stress memory in physiological, morphological and metabolic forms (Walter et al., 2013).
Stress-priming improves the accumulation of osmolytes through altered metabolic processes and these metabolites help in stress tolerance (abiotic stresses) during next growing season through revealing the preceding stress memory (Tabassum et al., 2017) acting as antioxidants, defense compounds and osmoregulators (Rivas-Ubach et al., 2012). Progeny of the stressed plants store more proline and glycine betaine than non-stressed plants (Tabassum et al., 2017). Plants tolerate abiotic stresses through adjustments in gene expression, soluble sugars, proline contents, higher antioxidant, and through by biosynthesis of stress proteins (Sung et al., 2003; Yamada et al., 2007).

During stress-priming, plants attain cross tolerance to successive stresses through improved gene expression for osmolytes and heat shock proteins by buildup of transcription factors (Kibinza et al., 2011; Chen et al., 2012) and during the transcriptional drought memory, RNA polymerase II is involved (Ding et al., 2012). Priming enhances photosynthesis, up regulate Rubisco activase, and Rubisco while decrease malondialdehyde contents in primed plants under moisture stress (Wang et al., 2014) as was observed in this study (Tables 4 and 5) protein was enhance and lipid peroxidation was reduced.

Drought stress caused delayed, erratic and poor seedling growth and stand establishment of chickpea (Table 2) due to decreased sugar metabolism, disturbed α-amylase activity, trehalose contents (Table 3), decreased leaf CO2 assimilation rate, PSII efficiency, chlorophyll contents (Table 4), oxidative damage (Table 5), and reduced uptake of mineral elements (Table 6). The poor seedling growth under drought stress occur due to the deregulation of elongating cells which occur owing to disruption of water flow to the elongating cells from xylem (Nomani, 1998; Farooq et al., 2009). Lack of moisture disturbs the growth by ceasing the cell elongation, expansion and mitosis (Nonami, 1998; Farooq et al., 2009).

Sugars are responsible for regulation of the expression of α-amylase gene (Yu et al., 1990) and decrease in sugars under drought stress (Table 3) might impact α-amylase activity as well as trehalose contents (Table 3) as trehalose protects the plants against abiotic stresses via acting as scavenger, stabilizing the cell membranes and ceasing the protein denaturation (Benaroudj et al., 2001). Decrease in α-amylase activity strongly impacts the carbohydrate metabolism which further influences supply of food to the germinating seedling on the account of inhibited seedling growth and stand under less water than optimum conditions (Farooq et al., 2017b). Starch mobilization in germinating seeds is initiated by α-amylase (Fincher, 1989) and it maintains water potential during germination process through the provision of solute sugars (Murtaza and Asghar, 2012) and carbon source for germinating seedling (Farooq et al., 2017b).

Nutrient uptake (specifically N) is reduced under drought stress, which led to the increased apoplastic abscissic acid concentration with result in stomatal closing due to xylem sap alkalinization (Liu et al., 2005). Under the moisture stress the accumulation of malondialdehyde contents (an index of oxidative stress) (Table 5) increases which damages the membranes.

The increase of total sugars, reducing sugars, trehalose contents, free proline, total soluble phenolics and decrease in malondialdehyde contents (Tables 3 and 5) along with drought-priming under water stress provides tolerance to the chickpea plants through maintaining specific leaf area (Table 2) increasing the leaf CO2 net assimilation rate, PSII efficiency and total chlorophyll contents (Table 4), as the accumulation of same in the plants lowers the osmotic potentials of cells which results in drawing the H2O into the tissues and cells; hence maintains the turgor, and carbon intake (Ludlow and Muchow, 1990; Subbarao et al., 2000), thus improves the plant growth and development.

However, increased buildup of trehalose, free leaf proline and total soluble phenolics (Table 3 and 5), under moisture stress protects plants from ROS damage (Farooq et al., 2009; Tabassum et al., 2017), with stabilization of biological membranes due to presence of aromatic ring in the soluble phenolics (Fair et al., 2015) and by scavenging oxidative damage in cells (Takahama and Oniki, 1997). Likewise, under stress conditions, plant accumulate more proline contents (Table 5), which helps in macromolecules stabilization (Zhu, 2002; Wahid and Close, 2007) and works as a store nitrogen and carbon and a sink for excess reducing (Zhu, 2002) hence imparts tolerance against numerous stresses (Farooq et al., 2009).

In conclusion, drought-priming-induced alteration in seed composition, buildup of free leaf proline, total soluble phenolics and trehalose contents improved the chickpea performance under drought stress by modulating the oxidative stress, germination metabolism, carbon assimilation, PSII efficiency and uptake of minerals.

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