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# DPPH radical scavenging activity and contents of $H_2O_2$, malondialdehyde and proline in determining salinity tolerance in chickpea seedlings

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The involvement of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and contents of $H_2O_2$, malondialdehyde (MDA) and proline was investigated in determining salinity tolerance among seedlings of thirty chickpea (*Cicer arietinum* L.) genotypes having different pedigrees. Chickpea genotypes, including cultivars and advanced lines were grown for 7 days under control and salt stress (50 mM NaCl) conditions. The genotypes showed differential response to salt stress in terms of growth, DPPH radical scavenging activity and contents of $H_2O_2$, MDA and proline in seedlings. On the basis of seedling growth, the genotypes having better performance under stress conditions had reduced levels of $H_2O_2$ and MDA contents, but increased levels of proline and DPPH radical scavenging activity. Stress tolerance index for these parameters was also determined. Agglomerative hierarchal clustering by Pearson correlation coefficient grouped the genotypes into two major clusters — MC I and MC II. MC II and A1-1 sub-cluster of MC-I comprised mainly of genotypes that showed higher stress resistance levels for the respective parameters in comparison to genotypes in other sub-clusters. Thus, it is possible to identify salt-tolerant genotypes on the basis of above parameters without a field trial.

**Keywords**: Chickpea, *Cicer arietinum* L., DPPH radical scavenging activity, Hydrogen peroxide, Malondialdehyde, Proline

Plant growth and productivity are adversely affected by various abiotic and biotic stress factors. Abiotic stresses indeed are the primary factors which reduce the average yield of most crops by more than 50%[1]. Salinity is one of the most important of these stresses which affect plant growth and limit crop productivity worldwide[2]. Salinity effects are more conspicuous in arid and semi-arid regions, where the salt content of the soil is naturally high and rainfall is insufficient for leaching the excess salt[3]. In irrigated lands, the problem gets aggravated by agricultural practices, such as irrigation that can cause water tables to rise and concentrate salts in the root zone[4]. Poor quality water for irrigation and poor drainage are the main reasons for increase in soil salinity in irrigated areas[5]. As a result, over 800 million hectares of land throughout the world are salt affected either by salinity or by the associated condition of sodicity[6].

Chickpea (*Cicer arietinum* L.) is one of the world's most important leguminous food crops, cultivated in an area of nearly 10 million hectares across the world and accounts for about 15% of the total pulse production[7]. It is a cool season crop, mostly grown in dry and semi-dry regions of the world. Salinity has become an important constraint for chickpea cultivation.

Plants respond to salt stress and acclimatize through various biochemical and physiological changes[8]. Salt stress leads to oxidative stress due to increased production of reactive oxygen species (ROS), such as superoxide anion ($^{\cdot}O_2^-$), $H_2O_2$ and hydroxyl radical ($^{\cdot}OH$)[1]. These ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to membranes, proteins and nucleic acids. They cause lipid peroxidation, protein denaturation and DNA damage, leading to cell death[9]. During optimal conditions, the balance between ROS formation and consumption is tightly controlled by a complex antioxidative defence system[10].

When the crop experiences stress conditions, there is an activation and/or modulation of the activities of antioxidant enzymes which lead to enhanced cellular protection[11]. Salinity has been reported to affect $H_2O_2$ scavenging enzymes, plant water status and membrane integrity in chickpea[12]. Salt stress tolerance depends upon the capacity to detoxify the stress damage[13]. Acclimation to stress conditions is generally achieved by maintaining lower levels of $H_2O_2$ content and reduced lipid peroxidation[14]. The salt tolerant plants

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*Abbreviations*: DPPH, 1, 1-diphenyl-2-picrylhydrazyl; MDA, malondialdehyde; ROS, reactive oxygen species; STI, stress tolerance index; TCA, trichloroacetic acid.

show reduced $H_2O_2$ content and decreased lipid peroxidation in contrast to sensitive ones[15]. The deleterious effects of salt stress can be alleviated by increased antioxidant activity and proline content of plants[16]. Accumulation of compatible solutes and activation of antioxidant system are the effective measures for inducing salt resistance in plants[17].

The information regarding the relative levels of salt tolerance and anti-oxidative responses of chickpea under salt stress is limited. Moreover, the biochemical parameters which have been depicted to be related to salt tolerance have been proposed after considering only a few genotypes at a time. There is, therefore, a need to explore the effect of salt stress on the anti-oxidative potential and growth parameters of a large number of chickpea genotypes, which could help in their characterization for salt tolerance.

In the present investigation, thirty chickpea genotypes having different genetic background and of unknown salinity behaviour have been taken and the effects of salt stress on seedling growth, DPPH radical scavenging activity and contents of $H_2O_2$, MDA and proline have been determined to explore salt tolerant genotypes. The effectual involvement of these biochemical parameters in salt tolerance is also studied.

## Materials and Methods

### Plant material and experimental conditions

Thirty chickpea genotypes, including cultivars and advanced lines, having different genetic background and pedigree[18] were procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The seeds were washed with water, surface-sterilized with 0.1% $HgCl_2$ and cultivated aseptically in 250 ml conical flasks on 0.8% agar. Salt stress was provided with 50 mM NaCl in the medium. The flasks were then kept in an incubator at 25 ± 1°C under 12 h dark/light conditions for 7 days. Growth parameters, DPPH radical scavenging activity, $H_2O_2$, MDA and proline contents were determined in all the thirty genotypes. Stress tolerance index (STI) was determined for all the thirty genotypes.

### Determination of root, shoot lengths and biomass of seedlings

The growth was monitored by measuring root and shoot lengths and fresh weights of seedlings after 7 days of germination. Fresh tissue (roots, shoots and cotyledons) was dried at 70°C till constant weight was observed for determination of dry weights.

### Extraction and estimation of DPPH radical scavenging activity, $H_2O_2$, MDA and proline

Roots, shoots and cotyledons (100 mg tissue) were crushed with 2 ml of methanol. Homogenate was centrifuged at 10,000 *g* for 20 min and the DPPH radical scavenging activity of the supernatant was determined[19].

Roots, shoots and cotyledons (500 mg tissue) were crushed with 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) in a pre-chilled pestle and mortar. Homogenate was centrifuged at 12,000 *g* for 20 min and $H_2O_2$ content was estimated in the supernatant[20]. Malondialdehyde (MDA) was extracted with 5% (w/v) TCA and measured using a thiobarbituric acid reaction[21]. Proline was extracted with 3% sulfosalicylic acid and estimated[22].

### Determination of stress tolerance index (STI) of chickpea genotypes

STI that used to measure the drought resistance level of genotypes was deduced by modifying the formula given in literature[23].

$$STI = (Y_S)\,(Y_N) / (Y_{\bar{N}})^2$$

where $Y_S$ = DPPH radical scavenging activity/content of $H_2O_2$, MDA or proline/seedling growth of a given genotype in the salt stress environment; $Y_N$ = DPPH radical scavenging activity/content of $H_2O_2$, MDA or proline/seedling growth of a given genotype in a non-stress environment; and $Y_{\bar{N}}$ = Mean DPPH radical scavenging activity/content of $H_2O_2$, MDA or proline/seedling growth of a given genotype in a non-stress environment.

Level of stress resistance was determined by using the median values of STI for each parameter studied. The genotypes having STI ≥ median values for proline content, DPPH radical scavenging activity, length and biomass of roots and shoots and ≤ median values for biomass of cotyledons and the contents of $H_2O_2$ and MDA were marked positive for stress resistance level.

### Data analysis

By applying XLSTAT 2012.6.09 software, hierarchical agglomerative clustering was done by Pearson correlation coefficient using flexible linkage.

## Results and Discussion

### Seedling growth

The chickpea genotypes showed differential response to salt stress in terms of seedling growth (Table 1). Seedling establishment is crucial for crop

Table 1—Effect of salt stress induced by 50 mM NaCl on seedling growth of chickpea genotypes at 7 days of germination
[Data represent mean ± SD per seedling of six seeds in three replicates. Values without parentheses are for control plants and those with parentheses are for stressed plants]

| Genotypes | Roots | | | Shoots | | |
|---|---|---|---|---|---|---|
| | Length (cm) | Fwt (mg) | Dwt (mg) | Length (cm) | Fwt (mg) | Dwt (mg) |
| RSG-811 | 2.73 ± 0.53 (3.80 ± 0.45) | 41.5 ±6.0 (70.0 ±2.0) | 14.5 ± 0.5 (9.0 ± 0.2) | 1.78 ± 0.08 (2.43 ± 0.12) | 45.0 ± 9.0 (68.0 ± 6.0) | 5.5 ± 0.5 (11.0 ±1.0 ) |
| GJG-515 | 3.65 ± 0.02 (2.77 ± 0.14) | 74.5 ± 1.0 (60.5 ± 2.0) | 5.5 ± 0.5 (7.5 ± 0.5) | 2.52 ± 0.28 (2.13 ± 0.13) | 52.5 ± 8.5 (50.5 ± 3.5) | 5.0 ± 0.1 (6.5 ± 0.5) |
| GL-22044 | 5.55 ± 0.45 (5.25 ± 0.08) | 112.0 ±13 (71.5 ± 5.0) | 12.0 ± 0.5 (5.5 ± 0.5) | 3.08 ± 0.18 (2.49 ± 0.33) | 58.5 ± 2.5 (59.0 ± 4.0) | 6.5 ± 2.5 (5.0 ± 1.0) |
| GL-28228 | 6.75 ± 0.25 (4.88 ± 0.03) | 164.0 ±8.0 (82.0 ± 6.0) | 12.0 ± 0.2 (6.5 ± 0.5) | 5.06 ± 0.31 (3.61 ± 0.56) | 124.0 ± 13 (86.0 ±7.0) | 11.5 ± 1.5 (9.5 ± 1.5) |
| GL-28157 | 5.28 ± 0.45 (5.69 ± 0.36) | 108.0 ± 16 (86.0 ±2.0) | 8.0 ± 0.2 (7.5 ± 0.5) | 3.99 ± 0.16 (2.65 ± 0.12) | 104.0 ± 7.0 (73.0 ± 6.0) | 11.0 ± 1.0 (8.0 ± 1.0) |
| GNG-1861 | 6.25 ± 0.48 (3.15 ± 0.17) | 130.5 ± 5 (59.5 ±2.0) | 13.5 ± 0.5 (10.5 ± 0.5) | 3.82 ± 0.52 (1.97 ± 0.01) | 71.5 ± 5.0 (53.0 ± 3.5) | 14.5 ± 0.5 (11.5 ± 1.5) |
| GL-26083 | 3.46 ± 0.21 (3.59 ± 0.13) | 76.0 ± 1.0 (53.5 ± 4.0) | 5.0 ± 0.1 (9.5 ± 0.5) | 2.92 ± 0.15 (1.83 ± 0.27) | 62.0 ± 4.0 (26.5 ± 2.5) | 7.5 ± 0.5 (3.0 ±0.1 ) |
| PDG-3 | 5.27 ± 0.23 (3.31 ± 0.13) | 91.0 ± 3.0 (55.0 ±1.0) | 14.0 ± 0.3 (5.5 ± 0.5) | 4.32 ± 0.03 (2.27 ± 0.23) | 80.5 ± 1.5 (41.5 ± 3.5) | 13.5 ± 2.5 (4.0 ± 0.1) |
| PDG-4 | 5.00 ± 0.13 (3.93 ± 0.10) | 116.5 ± 2.0 (52.5 ± 7.0) | 8.0 ± 0.2 (5.5 ± 0.5) | 3.64 ± 0.13 (2.72 ± 0.05) | 58.5 ± 2.5 (44.0 ± 1.5) | 6.5 ± 1.5 (5.0 ± 0.1) |
| GL-28152 | 3.34 ± 0.63 (2.58 ± 0.28) | 97.5 ± 3.0 (52.0 ± 8.0) | 5.5 ± 0.1 (5.0 ± 0.1) | 3.30 ± 0.37 (2.08 ± 0.13) | 54.0 ± 2.0 (48.5 ± 1.0) | 7.5 ± 1.5 (7.3 ± 1.5) |
| GL-27091 | 3.76 ± 0.46 (3.01 ± 0.31) | 109.0 ± 13 (52.0 ± 1.0) | 6.5 ± 0.5 (2.5 ± 0.5) | 3.63 ± 0.09 (2.40 ± 0.27) | 83 ± 5.0 (55.0 ± 2.0) | 7.0 ± 0.1 (6.5 ± 0.1) |
| GL-21107 | 5.07 ± 0.13 (3.85 ± 0.20) | 112.0 ± 2.0 (63.5 ± 5.0) | 8.0 ± 0.1 (7.0 ± 0.1) | 4.33 ± 0.40 (2.83 ± 0.03) | 87.0 ± 9.0 (47.0 ± 6.0) | 8.5 ± 0.5 (7.0 ± 0.2) |
| GPF-2 | 4.35 ± 0.10 (2.37 ± 0.12) | 64.5 ± 3.0 (41.5 ± 5.0) | 7.5 ± 0.5 (8.0 ± 0.1) | 3.13 ± 0.18 (2.53 ± 0.47) | 62.5 ± 2.5 (44.0 ± 5.0) | 7.5 ± 0.15 (7.5 ± 0.5) |
| GL-28164 | 2.55 ± 0.10 (3.15 ± 0.10) | 51.0 ± 2.0 (73.5 ± 4.0) | 7.5 ± 0.5 (11.0 ± 0.1) | 1.55 ± 0.05 (1.35 ± 0.25) | 43.0 ± 3.0 (45.0 ± 3.0) | 5.0 ± 0.1 (6.0 ± 2.0) |
| GL-28156 | 5.15 ± 0.42 (3.54 ± 0.03) | 165.5 ± 15 (66.0 ± 2.0) | 12.5 ± 0.5 (5.0 ± 0.1) | 3.86 ± 0.03 (2.68 ± 0.08) | 96.5 ± 3.5 (66.5 ± 2.5) | 9.5 ± 0.5 (7.5 ± 0.5) |
| GL-28137 | 5.58 ± 0.01 (3.86 ± 0.03) | 120.5 ± 7.0 (59.5 ± 6.0) | 8.5 ± 0.5 (7.5 ± 0.5) | 4.46 ± 0.02 (2.64 ± 0.14) | 90.0 ± 2.0 (61.5 ± 5.0) | 12.5 ± 0.5 (9.5 ± 0.5) |

Fwt, Fresh weight; Dwt, Dry weight

production under saline conditions, which affect various physiological and biochemical mechanisms associated with growth[24]. Roots are the prime organs of the plant which encounter salinity[25]. Root traits are, therefore, likely to be one of the most important components of salinity tolerance in chickpea. There was a significant decrease in root length (10-50%) of all the genotypes, except in the seedlings of RSG-811, GL-22044, GL-28157, GL-26083 and GL-28164, where it remained almost unaffected by salinity (Table 1). There was a 29-86% decrease in root dry weight of chickpea genotypes, except in GJG-515, GL-26083, GPF-2 and GL-28164, where root dry weight was significantly increased with GL-26083, exhibiting a 90% increase in dry weight under stress conditions.

Shoot length of seedlings also decreased significantly in all the genotypes, except RSG-811 that remained unaffected under stress conditions (Table 1). Dry biomass of shoots decreased in all the genotypes under stress conditions, except RSG-811, GJG-515, GL-285152, GPF-2 and GL-28164, where it was either unaffected or showed a significant increase in dry weight (Table 1).

Inhibition of root and shoot growth is a common response to salinity, but the extent to which seedlings can counteract the stress conditions depends upon the nature of the cultivar[26]. The reduction in root and shoot growth might be due to the toxic effects of the high levels of NaCl concentration[27]. Growth processes are especially salt-sensitive, so that growth rates and biomass production provide reliable criteria for

assessing the ability of a plant to withstand salinity[28]. Longer and stronger root and shoot development will allow more successful selection for salt tolerance[29]. Accordingly, the genotypes GJG-515 and GL-28164 exhibiting increased shoot and root dry biomass under salt stress conditions had higher stress tolerance capacity. These genotypes exhibited relatively lower shoot dry biomass under control conditions. It may be inferred that genotypes having reduced shoot biomass under non-saline conditions would be more tolerant to salt stress conditions. Similar relation between biomass and salt tolerance in chickpea is also reported earlier[30].

**DPPH radical scavenging activity**

Antioxidant defence system plays an important role in plant's response to stress conditions. It protects the plants from oxidative damage to biomolecules. DPPH radical scavenging activity is a measure of non-enzymatic antioxidant activity[31]. DPPH radical scavenging activity increased significantly in the roots of PDG-3, PDG-4, GL-28228 and GL-28137 seedlings under salt stress (Fig. 1). Higher levels of DPPH radical scavenging activity in radicles have been correlated with enhanced stress tolerance in rice and cucumber seedlings[31,32]. The shoots of PDG-3 and GL-28228 also exhibited higher DPPH radical scavenging activity, as compared to other genotypes, under stress conditions (Fig. 1).

**$H_2O_2$ and MDA contents**

$H_2O_2$ is beneficial at low concentrations, but an excess is harmful to the plants[33]. At optimal concentrations, it performs various functions in the development, metabolism and homeostasis of aerobic organisms[34]. At high concentrations, it is injurious to plants, resulting in lipid peroxidation and membrane injury[35]. The accumulation of ROS is reported to be sensed as an 'alarm' signal that initiates pre-emptive defence responses[36].

There was reduced concentration of $H_2O_2$ in roots of GJG-515, PDG-4, GPF-2 and RSG-811 seedlings under salt stress (Fig. 2). The shoots of GJG-515, PDG-4, GL-28156 and GPF-2 seedlings also showed reduced $H_2O_2$ levels under stress conditions (Fig. 2). The reduced concentration of $H_2O_2$ in roots and shoots of PDG-4 showed effective correlation with its enhanced antioxidant capacity in terms of DPPH radical scavenging activity (Fig. 1). Among the sixteen genotypes showing effective salt tolerance, GL-22044, GL-28152, GL-27091, GL-28137 and GL-28157 showed significant increase in $H_2O_2$ content in roots and shoots of seedlings under salt stress (Fig. 2). A higher accumulation of $H_2O_2$ has been reported in the roots of *Brassica oleracea* under salt stress conditions[25].

Salinity is known to result in extensive lipid peroxidation, which is used as an indicator of stress-induced oxidative damage to membranes[37].

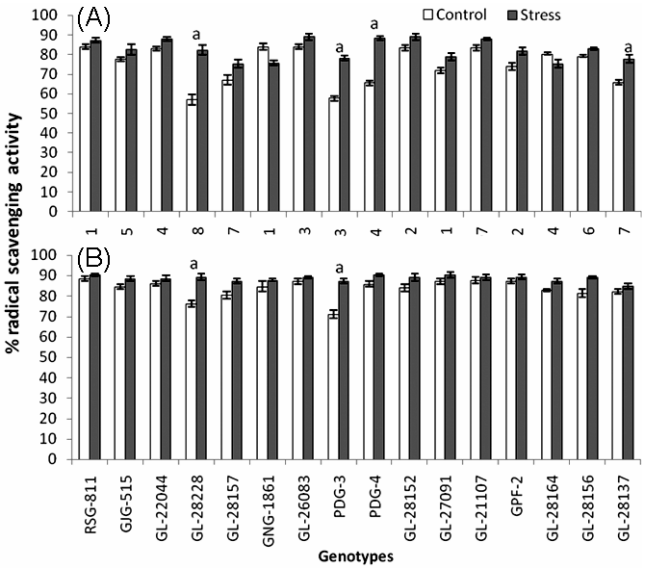


Fig. 1—Effect of salt stress on DPPH radical scavenging activity in roots (A) and shoots (B) of chickpea genotypes at 7 days of germination [Differences significant in comparison with respective controls at [a] – $P < 0.01$ (Student's t- test)]

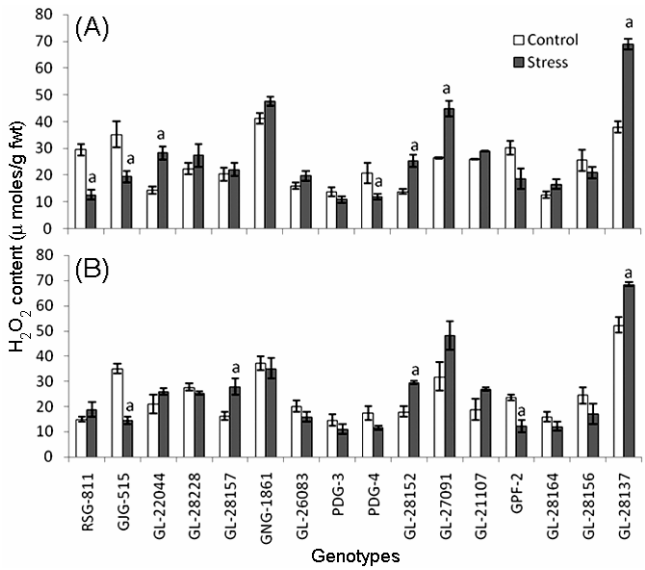


Fig. 2—Effect of salt stress on $H_2O_2$ content (µmoles/g fwt) in roots (A) and shoots (B) of chickpea genotypes at 7 days of germination [Differences significant in comparison with respective controls at [a] – $P < 0.01$ (Student's t- test)]

ROS like peroxides of polyunsaturated fatty acids on decomposition generate malondialdehyde (MDA) which is the most abundant individual aldehydic lipid breakdown product[38]. MDA is a widely used marker for evaluating oxidative lipid injury and its concentration varies in response to abiotic stresses[39]. MDA content was observed to be lower in the roots and shoots of PDG-3, PDG-4, GJG-515 and RSG-811 seedlings under salt stress conditions (Fig. 3). It is reported that the rate of lipid peroxidation (in terms of MDA content) indicates the sensitivity of plants to salt stress[40]. The decreased MDA content in shoots/roots of PDG-3, PDG-4, GJG-515 and RSG-811 indicated reduced oxidative damage to their membranes and thereby attributed to their stress tolerant behaviour. On the other hand, certain genotypes, e.g., PG-00110, GL-26083 and GNG- 1958 that showed increased MDA content in their seedlings (data not given) showed extensive lipid peroxidation which revealed their salt stress susceptible nature.

The reduced contents of $H_2O_2$ and MDA in salt-tolerant plants are reported in literature[15]. An effective relation was observed between MDA and $H_2O_2$ contents with DPPH radical scavenging activities of genotypes under salt stress. The genotypes exhibiting better performance under saline conditions had reduced levels of MDA (Fig. 3) which correlated well with their decreased $H_2O_2$ contents (Fig. 2) and increased DPPH radical scavenging activities (Fig. 1). These genotypes thus achieved better stress tolerance by reducing lipid peroxidation of membrane systems through an enhanced antioxidant capacity. The genotypes exhibiting stress intolerance had higher levels of MDA in their seedlings which might result from lack of salt-dependent upregulation of anti-oxidative system as described elsewhere[41].

**Proline content**

Plants can partly protect themselves against abiotic stresses by accumulating compatible solutes, which can stabilize proteins and cellular structures[42]. Proline is one of the most common compatible osmolyte which maintains the osmotic potential. It also maintains redox metabolism by removing excess levels of ROS and re-establishing cellular redox balance[43]. In addition to its ROS scavenging activity, proline is also reported to protect and stabilize ROS scavenging enzymes and activate alternative detoxification pathways in plants subjected to various abiotic stresses[44]. Proline, therefore, acts both as a direct antioxidant, as well as an activator of mechanisms that act as antioxidants.

Chickpea genotypes that performed better under salt stress conditions were observed to accumulate higher levels of proline in their seedlings, as compared to those which could not tolerate the harsh saline conditions (Fig. 4).

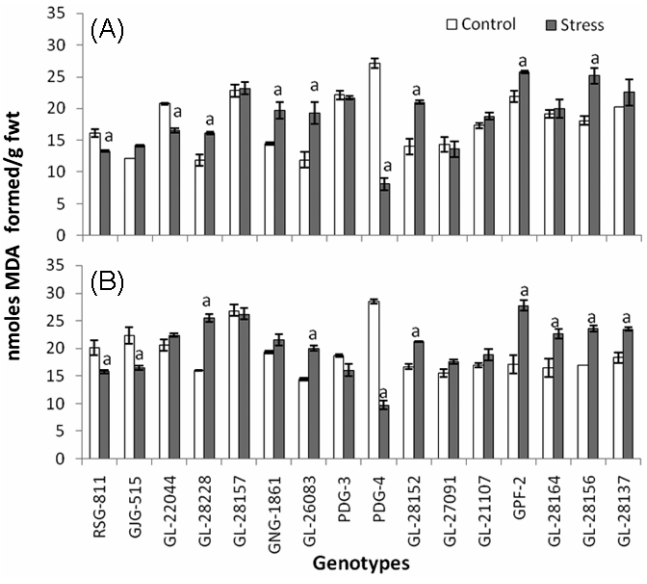


Fig. 3—Effect of salt stress on MDA content (nmoles MDA formed/g fwt) in roots (A) and shoots (B) of chickpea genotypes at 7 days of germination [Differences significant in comparison with respective controls at [a] – $P < 0.01$(Student's t- test)]

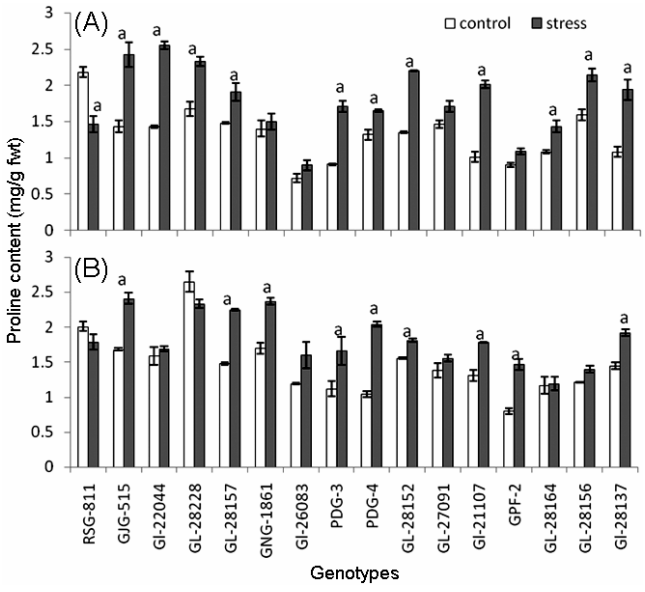


Fig. 4—Effect of salt stress on proline content (mg/g fwt) in roots (A) and shoots (B) of chickpea genotypes at 7 days of germination [Differences significant in comparison with respective controls at [a] – $P < 0.01$(Student's t- test)]

Among the sixteen genotypes showing effective salt tolerance, the roots of GJG-515, PDG-3, GL-21107, GL-22044 and GL-28152 showed ~2-fold increase in proline content under stress conditions (Fig. 4). Salt stress has been reported to enhance the expression of pyrroline-5-carboxylate synthase 1 (P5CS1) in the roots of tolerant canola line[45]. Proline content was also observed to be ~1.5 to 2-fold higher in the shoots of PDG-4, GPF-2 and GJG-515 seedlings under stress conditions (Fig. 4). The content, in fact, decreased in some genotypes which showed salt intolerance (data not given).

Accumulation of proline, therefore, acted as an adaptive mechanism of salt tolerance, which protected the relatively tolerant genotypes against salt-induced oxidative stress. Proline accretion in plants subjected to various abiotic stresses has been reported earlier[8]. It is thus proposed that chickpea genotypes which adopted physiological adaptive mechanisms to regulate their redox status performed better under saline conditions, as compared to those which could not acquire these mechanisms.

**Stress tolerance index (STI) and stress resistance levels**

Based on STI for the various parameters (Table 2), the level of stress resistance was calculated for all the thirty genotypes. The genotypes having STI ≥ median values for proline content, DPPH radical scavenging activity, length and biomass of roots and shoots and those with STI ≤ median values for biomass of cotyledons and the contents of $H_2O_2$ and MDA were marked positive for stress resistance level (Table 2). The genotypes viz., RSG-811, GJG-515, PBG-1, GL-22044, GL-28228, GL-28157, GNG-1861, PDG-4, GL-27091, GL-21107, GL-26083, PDG-3 and GL-28152 having STI ≥ median values for most of the parameters studied (eg. DPPH radical scavenging activity, proline content, root length, shoot length and their biomass) or ≤ median values for $H_2O_2$ and MDA contents and fresh and dry biomass of cotyledons were proposed to have higher stress resistance levels. Similarly, the genotypes PG-97030, PG-00110, GNG-469, GNG-1958, GNG-1581 and GG-1362 were proposed to be highly susceptible on the basis of their lower stress resistance level, as compared to other genotypes.

**Statistical analysis**

Agglomerative hierarchal clustering by Pearson correlation coefficient using flexible linkage categorized the thirty genotypes on similarity basis into two major clusters, viz. MC-I and MC-II. MC-I, being a large cluster comprised 24 genotypes, whereas MC-II contained only six genotypes (Fig. 5). The larger cluster MC-1 was divided into two sub-clusters, viz., A and B. The 'A' sub-cluster was

Table 2—Stress tolerance index of various parameters in chickpea seedlings

| Genotype | Roots | | | | | | | Shoots | | | | | | | Cotyledons | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lth | Fwt | Dwt | Pro | $H_2O_2$ | MDA | DPPH | Lth | Fwt | Dwt | Pro | $H_2O_2$ | MDA | DPPH | Fwt | Dwt | Pro | $H_2O_2$ | MDA | DPPH |
| NDG-9-21 | 0.48 | 0.60 | 2.76 | 2.75 | 1.19 | 1.04 | 1.16 | 0.26 | 0.10 | 0.72 | 1.07 | 1.06 | 3.01 | 1.09 | 1.74 | 1.58 | 1.19 | 3.97 | 1.53 | 1.37 |
| PG-97030 | 0.06 | 0.08 | 0.47 | 0.49 | 1.99 | 1.17 | 1.12 | 0.09 | 0.23 | 0.34 | 0.73 | 1.34 | 1.09 | 0.97 | 1.64 | 1.56 | 1.48 | 2.54 | 0.96 | 1.58 |
| GL-28170 | 0.49 | 0.51 | 0.46 | 0.80 | 1.65 | 0.97 | 1.21 | 0.54 | 0.44 | 0.77 | 0.72 | 0.72 | 2.02 | 1.04 | 0.76 | 0.90 | 0.70 | 3.03 | 1.04 | 1.07 |
| PG-00110 | 0.42 | 0.35 | 0.41 | 2.62 | 1.92 | 1.76 | 1.23 | 0.28 | 0.42 | 0.54 | 1.24 | 0.92 | 1.90 | 1.04 | 1.95 | 1.97 | 2.21 | 1.81 | 1.19 | 1.45 |
| GNG-469 | 0.51 | 0.48 | 0.49 | 0.63 | 1.08 | 1.92 | 0.94 | 0.60 | 0.79 | 0.67 | 1.04 | 2.60 | 2.39 | 1.00 | 1.74 | 1.74 | 1.26 | 2.89 | 2.05 | 0.94 |
| BGM-569 | 0.64 | 0.63 | 0.71 | 1.44 | 2.27 | 1.02 | 1.02 | 0.75 | 0.68 | 0.82 | 1.22 | 1.64 | 1.07 | 0.93 | 1.11 | 0.81 | 1.04 | 2.10 | 0.92 | 0.76 |
| GNG-1581 | 0.51 | 0.33 | 0.40 | 0.85 | 1.48 | 1.77 | 1.02 | 0.76 | 0.82 | 0.53 | 1.12 | 0.73 | 1.53 | 0.94 | 0.64 | 0.68 | 0.60 | 3.50 | 1.02 | 0.98 |
| GNG-1958 | 0.87 | 0.57 | 0.09 | 0.91 | 1.08 | 1.92 | 1.19 | 0.31 | 0.43 | 1.04 | 1.30 | 2.37 | 1.92 | 1.08 | 1.73 | 2.15 | 0.76 | 2.23 | 1.71 | 0.81 |
| RSG-811 | 0.61 | 0.32 | 1.76 | 1.73 | 0.42 | 0.64 | 1.32 | 0.42 | 0.61 | 0.78 | 1.62 | 0.29 | 0.68 | 1.17 | 2.55 | 2.94 | 1.27 | 1.35 | 0.48 | 1.13 |
| GJG-515 | 0.59 | 0.49 | 0.56 | 1.88 | 0.77 | 0.51 | 1.16 | 0.52 | 0.52 | 0.42 | 1.82 | 0.53 | 0.78 | 1.10 | 0.70 | 0.75 | 1.69 | 1.00 | 0.62 | 1.10 |
| GL-28184 | 0.53 | 0.45 | 0.71 | 2.35 | 0.40 | 0.64 | 1.08 | 0.45 | 0.42 | 0.29 | 2.15 | 0.32 | 0.70 | 1.03 | 0.95 | 1.02 | 2.06 | 0.88 | 0.83 | 0.80 |
| PBG-1 | 2.21 | 1.16 | 0.53 | 2.23 | 1.11 | 0.88 | 0.95 | 1.56 | 1.96 | 0.92 | 1.64 | 1.17 | 0.58 | 0.89 | 0.58 | 0.61 | 0.87 | 0.54 | 0.82 | 0.64 |
| GL-22044 | 1.70 | 0.88 | 0.89 | 1.98 | 0.46 | 1.02 | 1.32 | 0.75 | 0.68 | 0.42 | 1.21 | 0.57 | 0.98 | 1.12 | 0.99 | 1.64 | 1.32 | 0.64 | 1.20 | 1.98 |

*(Contd.)*

Table 2—Stress tolerance index of various parameters in chickpea seedlings—(*Contd.*)

| Genotype | Roots | | | | | | | Shoots | | | | | | | Cotyledons | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lth | Fwt | Dwt | Pro | $H_2O_2$ | MDA | DPPH | Lth | Fwt | Dwt | Pro | $H_2O_2$ | MDA | DPPH | Fwt | Dwt | Pro | $H_2O_2$ | MDA | DPPH |
| GL-28228 | 1.93 | 1.47 | 1.05 | 2.11 | 0.69 | 0.57 | 0.85 | 1.78 | 2.11 | 1.41 | 2.77 | 0.73 | 0.87 | 1.00 | 1.22 | 1.06 | 1.67 | 0.61 | 1.34 | 1.47 |
| GL-28157 | 1.76 | 1.02 | 0.81 | 1.53 | 0.51 | 1.57 | 0.91 | 1.03 | 1.50 | 1.14 | 1.50 | 0.47 | 1.50 | 1.03 | 1.56 | 1.80 | 1.16 | 0.65 | 0.95 | 2.31 |
| PBG-5 | 0.48 | 0.49 | 1.01 | 1.09 | 1.09 | 1.24 | 1.24 | 0.61 | 1.03 | 1.21 | 1.60 | 1.42 | 1.07 | 0.93 | 0.97 | 1.35 | 1.68 | 0.95 | 1.35 | 0.91 |
| GNG-1861 | 1.15 | 0.85 | 1.92 | 1.14 | 2.20 | 0.85 | 1.15 | 0.74 | 0.75 | 2.15 | 1.81 | 1.36 | 0.88 | 1.09 | 0.75 | 0.54 | 1.52 | 0.83 | 0.79 | 1.07 |
| GL-26083 | 0.73 | 0.45 | 0.64 | 0.35 | 0.36 | 0.68 | 1.35 | 0.52 | 0.32 | 0.29 | 0.86 | 0.33 | 0.61 | 1.14 | 0.65 | 0.67 | 0.74 | 0.44 | 0.94 | 1.11 |
| PDG-3 | 1.02 | 0.55 | 1.04 | 0.84 | 0.17 | 1.42 | 0.82 | 0.96 | 0.66 | 0.70 | 0.84 | 0.17 | 0.64 | 0.91 | 0.45 | 0.45 | 0.81 | 0.28+ | 0.97 | 0.98 |
| PDG-4 | 1.15 | 0.67 | 0.59 | 1.18 | 0.28 | 0.65 | 1.05 | 0.97 | 0.51 | 0.42 | 0.95 | 0.21 | 0.59 | 1.14 | 0.39 | 0.34 | 0.85 | 0.46 | 0.71 | 1.25 |
| GL-28152 | 0.50 | 0.55 | 0.37 | 1.61 | 0.40 | 0.87 | 1.34 | 0.67 | 0.52 | 0.71 | 1.27 | 0.55 | 0.75 | 1.10 | 0.60 | 0.54 | 1.28 | 1.25 | 1.12 | 0.82 |
| GL-27091 | 0.66 | 0.62 | 0.22 | 1.35 | 1.34 | 0.58 | 1.02 | 0.85 | 0.90 | 0.59 | 0.97 | 1.60 | 0.58 | 1.16 | 0.66 | 0.85 | 1.23 | 0.86 | 0.97 | 1.06 |
| GL-21107 | 1.14 | 0.78 | 0.76 | 1.10 | 0.85 | 0.96 | 1.32 | 1.20 | 0.81 | 0.77 | 1.05 | 0.53 | 0.68 | 1.15 | 0.59 | 0.57 | 1.09 | 1.02 | 1.03 | 0.96 |
| GPF-2 | 0.60 | 0.29 | 0.81 | 0.53 | 0.64 | 1.68 | 1.09 | 0.77 | 0.54 | 0.73 | 0.53 | 0.30 | 1.01 | 1.14 | 0.66 | 0.71 | 0.77 | 1.44 | 0.91 | 0.72 |
| GL-28164 | 0.47 | 0.41 | 1.12 | 0.84 | 0.24 | 1.14 | 1.10 | 0.20 | 0.38 | 0.39 | 0.63 | 0.20 | 0.79 | 1.06 | 1.23 | 0.93 | 0.89 | 1.03 | 0.96 | 0.93 |
| GL-28156 | 1.06 | 1.20 | 0.85 | 1.84 | 0.60 | 1.35 | 1.19 | 1.01 | 1.27 | 0.92 | 0.76 | 0.44 | 0.85 | 1.07 | 1.12 | 2.10 | 1.52 | 1.06 | 1.13 | 1.30 |
| GL-28137 | 1.26 | 0.78 | 0.86 | 1.14 | 2.95 | 1.35 | 0.93 | 1.15 | 1.09 | 1.53 | 1.25 | 3.72 | 0.92 | 1.02 | 0.79 | 0.73 | 0.45 | 0.36 | 1.14 | 0.82 |
| GL-27104 | 0.53 | 0.61 | 0.24 | 1.23 | 4.24 | 1.20 | 1.04 | 0.57 | 0.72 | 1.47 | 0.70 | 5.53 | 1.24 | 1.02 | 0.88 | 0.91 | 0.59 | 0.36 | 1.02 | 0.66 |
| GL-26054 | 0.93 | 0.76 | 1.21 | 0.94 | 3.73 | 1.21 | 0.93 | 0.78 | 0.42 | 0.21 | 0.72 | 4.45 | 1.19 | 1.19 | 0.62 | 0.67 | 0.66 | 0.56 | 0.92 | 1.15 |
| GG-1362 | 0.88 | 1.44 | 1.01 | 0.86 | 4.98 | 1.22 | 1.01 | 0.66 | 0.62 | 0.61 | 0.71 | 6.56 | 0.82 | 1.06 | 1.07 | 1.05 | 0.73 | 0.23 | 1.04 | 0.81 |

Lth, Length; Fwt, Fresh weight; Dwt, Dry weight; Pro, Proline

further divided into A1 and A2 clusters. The A1 cluster was further subdivided into A1-1 and A1-2 clusters. The A1-1 cluster mainly comprised genotypes having higher stress resistance levels, except GL-28164 and PBG-5, which exhibited lower stress tolerance index (Fig. 5; Table 2). These genotypes also exhibited reduced $H_2O_2$ content in either roots or shoots under salt stress conditions (Fig. 2). The A1-2 and A2 clusters comprised of genotypes exhibiting lower stress tolerance index (Fig. 5; Table 2) and hence exhibited poor performance under salt stress conditions. The 'B' cluster of MC-1 comprised genotypes exhibiting moderate to high levels of STI, except GG-1362 and GL-27104 (Fig. 5; Table 2).

The six genotypes present in MC-II had higher STI for most of the parameters studied (Fig. 5; Table 2). Most of the genotypes in this cluster exhibited decreased content of $H_2O_2$ in roots and shoots under stress conditions (Fig. 2). The major cluster MC-II of the dendrogram exclusively comprised genotypes exhibiting higher stress resistance levels for most of the biochemical and physiological parameter studied (Fig. 5).

It was observed that most of the genotypes present in MC-II and A1-1 cluster of MC-I had higher DPPH radical scavenging activity coupled with relatively lower $H_2O_2$ and MDA contents (Fig. 5). On the contrary, the genotypes exhibiting very low DPPH radical scavenging activity together with relatively higher $H_2O_2$ and MDA contents under stress conditions occupied unique positions in the A2 and A1-2 clusters of MC-1 (Fig. 5).

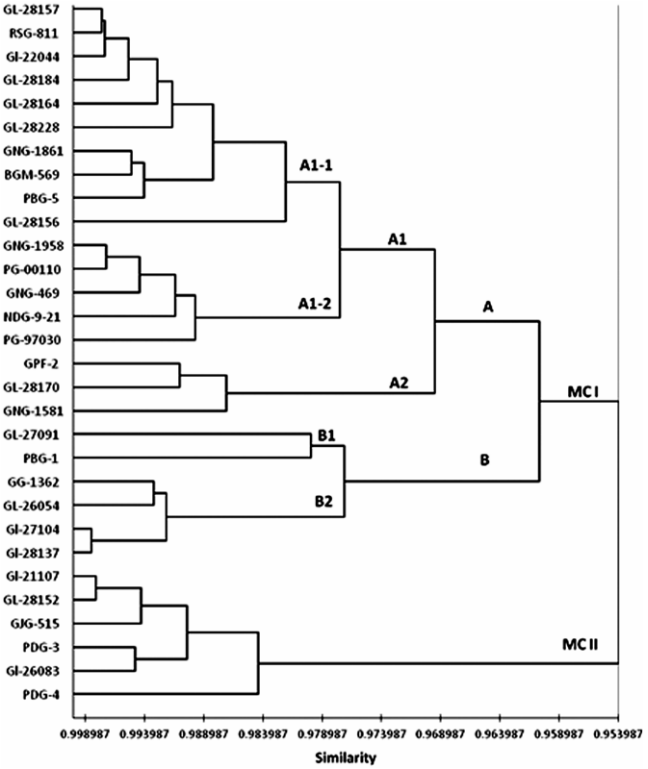


Fig. 5—Dendrogram of 30 chickpea genotypes obtained with Pearson correlation coefficient using flexible linkage [MC I, major cluster I; MC II, major cluster II; A and B, sub clusters of MC I; A1 and A2, clusters of A; A1-1 and A1-2, clusters of A1; B1 and B2, clusters of B]

Accordingly, most of the genotypes present in A1-1 and B clusters of MC-I and those exclusively present in MC-II constituted the salt stress-tolerant group, whereas the genotypes present in A1-2 and A2 clusters of MC-I constituted the salt-susceptible group, owing to their lower stress resistance levels for most of the biochemical and growth parameters.

## Conclusion

The study demonstrated that chickpea genotypes GNG-1861, RSG-811, GJG-515, GL-22044, GL-28228 and GL-28137 which exhibited improved root and shoot development system, enhanced DPPH radical scavenging activity, increased proline content and reduced levels of $H_2O_2$ and MDA under salt stress conditions were relatively more tolerant, as compared to those having lower stress resistance levels for these parameters.

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