Physiological Characterization of Elite Chickpea (Cicer arietinum L.) Genotypes for Salinity Stress Tolerance

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Received: 2.07.2020 | Revised: 8.08.2020 | Accepted: 13.08.2020

ABSTRACT
In this present investigation, the 12 elite chickpea genotypes characterized and classified into different classes based on physiological traits. Salinity stress reduced the relative water contents of leaves (RWC), membrane stability index (MSI), chlorophyll a and b contents, whereas, increased the relative stress injury (RSI) due to ionic and osmotic effects imposed by salt stress. Relative water contents and relative stress injury considered as a biomarker for selection of chickpea genotypes. Based on both the traits, genotypes viz., H 12-22, H 13-01, H 13-09, H 14-04, H 14-11, H 14-22 and H 15-05 had performed better in salinity stress chickpea field as compared to normal sown chickpea field. Moreover, a set of five important physiological traits included in the comparative ranking of chickpea genotypes with maximum times of higher ranks identified chickpea genotype H 13-01, H 14-22 and H 15-06 followed by H 14-04 and RSG 931. Therefore, genotypes H 13-01, H 14-22 and H 15-06 identified for salinity tolerance in the present study based on both biomarker analysis and comparative study which could be utilized for further improvement in salinity tolerance in chickpea germplasm for the development of elite breeding materials and improved chickpea varieties in future breeding programme.

Keywords: Chickpea, NaCl, RWC, RSI, Chlorophyll, MSI.

INTRODUCTION
Chickpea (Cicer arietinum L.) considered as the first grain legume to be domesticated by human, commonly known as gram or Bengal gram (Nagaroje et al., 2016). It is an important food legume crop after common bean-Phaseolus vulgaris L., & field pea- Pisum sativum L. (Aggarwal et al., 2015). It is widely cultivated crop in throughout Asian, European, Ethiopian, African and Australian continents as well as broadly distributed all over the tropics, subtropics and temperate regions (Gaur et al., 2008; FAO, 2013; ICRISAT, 2013; Rasool, 2013).

Cite this article: Janghel, D.K., Chhabra, A.K., Kumar, N., Devi, S., & Mor, V.S. (2020). Physiological Characterization of Elite Chickpea (Cicer arietinum L.) Genotypes for Salinity Stress Tolerance, Ind. J. Pure App. Biosci. 8(4), 228-236. doi: http://dx.doi.org/10.18782/2582-2845.8277
Among six major chickpea producing countries, India is chief producer contributed about 90% of the global chickpea production during 2018, include six countries, i.e., India (66.09 %), Australia (5.80%), Turkey (3.66%), Russia (3.60 %), United States of America (3.36 %) and Ethiopia (2.99 %), respectively in the world (FAO, 2020). Two types of chickpea identified based on seed characteristics in cultivated chickpea as Desi and Kabuli types. It is considered as a primary source of carbohydrates and proteins, which collectively constitute 80 % of the total dry seed weight (Talebi et al., 2008; Aggarwal et al., 2013). Chickpea is a salt sensitive species with an estimated worldwide yield loss of 8–10 % due to salinity and complete crop failure can occur in the worst affected soils (Flowers et al., 2010). An estimated area of total cultivated lands affected by soil salinity was 20 % (45 Mha) and global irrigated agricultural lands by 33 % (55.7 Mha) with increasing at alarming annual rate of 10 % for various reasons including low precipitation, high evaporation, alternate wet and dry season, saline irrigation water and poor drainage (Shrivastava & Kumar, 2015). Jamil et al. (2011) and Ladeiro (2012) reported that more than 50 % of global arable land would be affected by soil salinity by 2050. Salinity adversely affects many physiological processes in chickpea especially photosynthesis, chlorophyll contents and cell membrane stability which ultimately reducing chickpea seed yield (Chaves et al., 2009). The development of stress tolerant chickpea cultivars is currently one of the major challenges for the researchers (Garg et al., 2016). Therefore, characterization of elite chickpea genotypes for salinity stress tolerant is taken as objective of present study which is the basic need of any breeding programme.

MATERIALS AND METHODS

The experimental material comprised of 12 Desi chickpea genotypes taken from chickpea germplasm maintained at Pulses Section of the Department of Genetics and Plant Breeding, C.C.S. Haryana Agricultural University, Hisar (Table 1). These chickpea genotypes were grown in Randomized Block Design (RBD) with three replications under naturally existing salinity stress field conditions and normal field condition at Research Farm of Pulses Section, Department of Genetics and Plant Breeding during Rabi 2018-19 in single row plot of 2 m length for each genotype. All the recommended package of practices was followed for raising the good crop. The data for five physiological traits were recorded in 12 chickpea genotypes on five randomly selected plants (excluding border plants) from in each replication to assess the genetic diversity for salinity stress tolerance on chickpea. The physiological traits were included viz., relative water contents, chlorophyll a contents, chlorophyll b contents, relative stress injury and membrane stability index.

1. Leaf relative water content (RWC) was estimated according to Kumar and Elston (1992) using the equation: RWC (\%) = (Fresh mass – Dry mass) / (Maximum mass – Dry mass) x 100

2. In terms of relative stress injury (RSI %) measured as the EC of the external medium by Sullivan and Ross (1979): RSI (\%) = EC₁ / EC₂ x100

3. The cell membrane stability index (MSI) was calculated according to Sairam et al. (2002) as follows: MSI = [1- (EC₁ / EC₂)] x100

4. Chlorophyll and carotenoid contents were estimated using destructive method (DMSO) according to the method of Hiscox and Israelstam (1979).

\[ \text{Chl 'a' (mg/g tissue)} = 12.7 \ A_{663} - 2.69 \ A_{645} \times \frac{V}{1000 \times W} \]

\[ \text{Chl 'b' (mg/g tissue)} = 22.9 \ A_{645} - 4.68 \ A_{663} \times \frac{V}{1000 \times W} \]

Where, \(A\) = Absorbance at specific wavelengths, \(V\) = Final volume of chlorophyll extract in DMSO and \(W\) = Fresh weight of tissue extracted.
The data for different physiological traits of field experiment in RBD (randomized block design) were statistically analyzed as described by Gomez and Gomez (1984) by using Online Statistical Analysis Package (OPSTAT: http://14.139.232.166/opstat/, Computer Section, CCS Haryana Agricultural University, Hisar, India). The means of chickpea genotypes were compared by LSD (Least significance difference) at 5 % level of significance.

RESULTS AND DISCUSSION
The one way ANOVA due to genotypes in randomized block design (RBD) compared the 12 chickpea genotypes in three replications evaluated at Hisar in naturally existing salinity field and normal sown chickpea field during Rabi 2018-19. The mean sum of square (MSS) due to genotypes for various physiological traits viz., relative water contents (RWC %), relative stress injury (RSI %), membrane stability index (MSI %), chlorophyll-a and chlorophyll-b contents had found highly significant at 1 % level of significance in both naturally existing salinity stress chickpea field (Table 1) and normal sown chickpea field experiments during Rabi 2018-19 (Table 2) which indicated that all 12 chickpea genotypes were significantly different for various physiological traits.

Table 1: List of thirteen chickpea genotypes selected for physiological characterization

| Sr. No. | Name of chickpea genotypes | Pedigree | Developed by |
|---------|-----------------------------|----------|--------------|
| 1.      | H 12-22                     | HC 5 x H 00-256 | CCSHAU, Hisar |
| 2.      | H 12-29                     | HC 1 x (HC 1 x ICCV 96030) | CCSHAU, Hisar |
| 3.      | H 12-62                     | (HC 5 x H 00-256) x ICC 4958 | CCSHAU, Hisar |
| 4.      | H 12-63                     | (HC 5 x H 00-256) x ICC 4958 | CCSHAU, Hisar |
| 5.      | H 13-01                     | HC 5 x H 04-31 | CCSHAU, Hisar |
| 6.      | H 13-09                     | (HC 5 x H 00216) x H 208 | CCSHAU, Hisar |
| 7.      | H 14-01                     | HC 1 x H 04-31 | CCSHAU, Hisar |
| 8.      | H 14-04                     | H 03-56 x H 04-31 | CCSHAU, Hisar |
| 9.      | H 14-11                     | (HC 5 x PDG 84-16) x (HC 5 x H 91-36) | CCSHAU, Hisar |
| 10.     | H 14-22                     | HC 5 x H 208 | CCSHAU, Hisar |
| 11.     | H 15-05                     | HC 5 x H 208 | CCSHAU, Hisar |
| 12.     | H 15-06                     | GNG 663 x HC 1 | CCSHAU, Hisar |

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Table 1: One-way ANOVA (analysis of variance) for various physiological traits of chickpea genotypes in normal sown chickpea field during 2018-19

| Source of variation | Degree of freedom | Mean sum of squares (MSS) |
|---------------------|-------------------|--------------------------|
|                     | (SV) (df)         | RWC  | RSI | MSI | CHL-a | CHL-b |
| Replications        | 2                 | 8.303 | 2.730 | 2.767 | 0.164 | 0.005 |
| Genotypes           | 11                | 18.733 | 15.117 | 15.121 | 0.084 | 0.003 |
| Error               | 22                | 27.456 | 5.934 | 5.934 | 0.020 | 0.003 |

*Significant at 5% level, **Significant at 1% level
RSI- relative stress injury, CHL-a- chlorophyll-a, CHL-b- chlorophyll-b, MSI- membrane stability index and RWC- relative water contents
RWC (%) of leaves in chickpea genotypes decreased in salinity stress chickpea field as compared to normal sown chickpea field (Fig. A). The less than 15 % reduction in MSI of chickpea genotypes observed in H 12-22 (14.57), H 12-62 (8.07), H 12-63 (11.08), H 13-01 (7.94), H 13-09 (8.11), H 14-04 (7.95), H 14-11 (8.54), H 14-22 (12.12) and H 15-05 (8.26) in salinity stress chickpea field (Table 3), whereas, RSI (%) of leaves in chickpea genotypes increased in salinity stress chickpea field as compared to normal sown chickpea field (Fig. B). The per cent increase in RSI of chickpea genotypes had shown less than 15 % increase in H 12-29 (13.25), H 12-22 (9.99), H 13-01 (12.27), H 13-09 (10.96), H 14-01 (14.33), H 14-04 (13.14), H 14-11 (13.96), H 14-22 (13.47), H 15-05 (12.71) and H 15-06 (12.86) in salinity stress chickpea field (Table 3). Similarly, MSI (%) of leaves in chickpea genotypes decreased in salinity stress chickpea field as compared to normal sown chickpea field (Fig. C). The per cent reduction in MSI of chickpea genotypes exhibited less than 15 % in only two genotypes viz., H 14-04 (5.13) and H 14-22 (14.40) in salinity stress chickpea field (Table 3). Chlorophyll a and chlorophyll b contents of leaves (mg/g DW of leaves) of chickpea genotypes similarly decreased in salinity stress chickpea field as compared to normal sown chickpea field (Fig. D & E, respectively). The chlorophyll a contents of chickpea genotypes revealed less than 15 % reduction in H 13-01 (5.40), H 14-22 (8.14), H 15-05 (11.18) and H 15-06 (4.67) in salinity stress chickpea field (Table 3). For chlorophyll b contents found less than 15 % reduction in genotypes viz., H 12-29 (1.29), H 13-01 (10.38), H 14-01 (8.39), H 14-11 (12.41), H 14-22 (8.21) and H 15-06 (5.25) in salinity stress chickpea field (Table 3).

Salinity stress reduced the RWC, MSI, chlorophyll a and b contents of leaves, whereas, increased RSI due to ionic and osmotic effects imposed by salt stress. RWC represents water status of plant which is responsible for maintenance of high turgor pressure, plant growth and development. It is adversely affected by salt imposition which could lead to decrease in water uptake and injury of root system. Chlorophyll pigment measures the photosynthetic capability of genotypes and generally used to quantify leaf senescence in salt-stressed plants. The reduction in chlorophyll content reduces the carbon fixation which ultimately affects the photosynthesis of genotype. A higher reduction in chlorophyll contents indicated the salt susceptible chickpea genotypes. Plasma membranes are the primary site of ion-specific salt injury and RSI measure the electrolyte leakage due to cell membrane injury. Therefore, electrolyte leakage from plasma membranes is reported as one of the most important selection criterion for identification of salt-tolerant plants. High relative leaf water content (RWC) and a lower electrolyte leakage could be used as biomarkers of membrane integrity and stability under salt stress condition. Based on both the traits following genotypes had performed better in salinity stress chickpea field H 12-22, H 13-01, H 13-09, H 14-04, H 14-11, H 14-22 and H 15-05 as compared to normal sown chickpea field. The

| Source of variation | Degree of freedom | Mean sum of squares (MSS) |
|---------------------|------------------|--------------------------|
| (SV)                | (df)             | RWC                      |
| Replications        | 2                | 14.247                   |
| Genotypes           | 11               | 2.735                    |
| Error               | 22               | 100.270                  |
|                     |                  | 0.096                    |
|                     |                  | 0.001                    |
|                     |                  | 6.836                    |
|                     |                  | 5.934                    |
|                     |                  | 11.418                   |
|                     |                  | 0.003                    |
|                     |                  | 0.001                    |

*Significant at 5% level, **Significant at 1% level

RSI- relative stress injury, CHL-a- chlorophyll-a, CHL-b- chlorophyll-b, MSI- membrane stability index and RWC- relative water contents

Table 2: One-way ANOVA (analysis of variance) for various physiological traits of chickpea genotypes in naturally existing salinity stress chickpea field during 2018-19

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above results are broadly in conformity with previous findings in chickpea reported by Soren et al. (2020); Atieno et al. (2017); Pushpavalli et al. (2015); Arefian et al. (2014); Turner et al. (2013); Vadez et al. (2012); Krishnamurthy et al. (2011); Vadez et al. (2007) and Singh (2004).

Table 3: Comparative study of ranks of elite chickpea genotypes based on physiological performance in salinity stress chickpea field as compare to normal sown chickpea field evaluated at Hisar during Rabi 2018-19

| Chickpea genotypes | RWC ** | RSI R* | MSI R** | CHL a R* | CHL b R* | Repeated ranks | Sum total |
|--------------------|--------|--------|---------|----------|----------|----------------|-----------|
| H 12-22            | 16.30  | 10     | 13.25   | 7        | 24.28    | 10             | 10        |
| H 12-29            | 14.57  | 10     | 9.99    | 5        | 19.46    | 7              | 9         |
| H 12-62            | 8.07   | 10     | 15.08   | 11       | 26.19    | 11             | 11        |
| H 12-63            | 11.08  | 10     | 16.30   | 12       | 27.47    | 12             | 12        |
| H 13-01            | 7.94   | 10     | 12.27   | 3        | 19.77    | 7              | 7         |
| H 13-09            | 8.11   | 10     | 10.96   | 2        | 19.77    | 6              | 9         |
| H 14-01            | 18.83  | 10     | 14.33   | 10       | 21.33    | 8              | 8         |
| H 14-04            | 7.95   | 10     | 13.14   | 6        | 5.13     | 1              | 5         |
| H 14-11            | 8.54   | 10     | 13.96   | 9        | 22.20    | 9              | 11        |
| H 14-22            | 12.12  | 10     | 13.47   | 8        | 14.40    | 2              | 5         |
| H 15-05            | 8.26   | 10     | 12.71   | 4        | 16.07    | 4              | 5         |
| H 15-06            | 17.27  | 10     | 12.86   | 5        | 15.73    | 3              | 11        |

* R- Rank  **%R- per cent reduction,  ***%I- per cent increase

![Percentage reduction in RWC](A)

![Percentage increase in RSI](B)
Fig. (A) Percentage reduction in RWC (%); Fig. (B) Percentage increase in RSI (%) and Fig. (C) Percentage reduction in MSI (%) of chickpea genotypes in salinity stress chickpea field as compare to normal sown chickpea field evaluated at Hisar during Rabi 2018-19

Fig. (D) Percentage reduction in chlorophyll a contents and Fig. (E): Percentage reduction in chlorophyll b contents of chickpea genotypes in salinity stress chickpea field as compare to normal sown chickpea field evaluated at Hisar during Rabi 2018-19

A set of five important physiological traits included in the comparative ranking of chickpea genotypes which directly or indirectly contributing to salinity stress tolerance. The top three ranks included and considered for physiological ranking of each genotypes. The highest rank given to those chickpea genotype which had shown to least reduction in traits (RWC, MSI, CHL a and CHL b) and least increment in trait (RSI) in

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salinity stress chickpea field as compared to normal sown chickpea field followed by lesser performed genotypes. Further, the chickpea genotypes obtained maximum number of higher ranks considered as top performing genotypes in salinity stress condition. Comparative study of important physiological traits in salinity stress chickpea field had identified salinity tolerant chickpea genotypes based on ranking of five physiological traits. The chickpea genotype H 13-01, H 14-22 and H 15-06 scored higher ranks followed by H 14-04 and RSG 931 based on physiological ranks. Therefore, genotypes H 13-01, H 14-22 and H 15-06 identified for salinity tolerance in the present study based on both biomarker analysis and comparative study which could be used for further improvement in chickpea genotypes for salinity tolerance in future breeding programme.

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