Comparative Study of the Effects of Salinity on Plant Growth, Nodulation, and Leghemoglobin Content in Kabuli and Desi Cultivars of Cicer arietinum (L.)

Varsha Mudgal\textsuperscript{1*}, Nidhi Madaan\textsuperscript{1}, Anurag Mudgal\textsuperscript{2}, Alka Singh\textsuperscript{3}, P. Kumar\textsuperscript{3}

\textsuperscript{1}Department of Biotechnology; \textsuperscript{2}Department of Mechanical Engineering College of Engineering and Technology, IFTM Campus, Moradabad, UP, India; \textsuperscript{3}Department of Botany, Hindu College, Moradabad, UP, India

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Abstract

This study was conducted to compare plant growth, nodulation and leghemoglobin content in Kabuli and Desi cultivars of chickpea (Cicer arietinum (L.)) under saline conditions. All the varieties expressed greater adverse effects on the plant height, dry weight of shoot and root at 30 and 60 DAS whereas the deleterious effects of the salinity minimized at 90 DAS. It is evident that variety BG-256 registered maximum inhibition at all the three growth stages (30, 60 and 90 DAS) as compared to varieties PUSA-939 and PUSA-1053. Relative growth rate (RGR) is high in PUSA-1053 (Kabuli tolerant), stands midway in PUSA-939 (Kabuli moderate tolerant) and is low in BG-256 (Desi sensitive). Maximum nodulation was reported in variety PUSA-1053 at all the growth stages and all salinity levels (4–16 EC) while minimum nodulation occurred in variety BG-256. The data clearly indicate that leghemoglobin content of the nodules increased at 60 DAS and declined thereafter. Kabuli tolerant variety (PUSA-1053) showed highest and Desi sensitive (BG-256) showed least leghemoglobin content at all the salinity levels from 4-16 EC and at all the growth stages from 30-90 DAS. The dry weigh of shoot and number of nodules were affected more adversely than nodule dry weight and leghemoglobin content. It indicates that Kabuli cultivars are more tolerant to saline stress and show less deleterious effects on nodules and nitrogen fixation efficiency.

Key Words: Cultivar, salinity, EC (electrical conductivity), leghemoglobin, DAS (days after sowing), nodulation.

Introduction

Salinity produces harmful effects on plant growth and yield. Permanent and complementary solution to minimize the deleterious effects of the salinity is to select and grow cultivars that can provide economic yield under saline conditions. Among legume crops, Chickpea ranks first in terms of area and production. It provides a high quality protein to the people of developing countries. It is usually grown mixed with wheat, barley, linseed and mustard. Selected cultivars of chickpea are Desi type with small and brown seed (nearly 90% area) and Kabuli type with bold and cream-colored seed (growing in 10% area). Desi chickpeas have markedly higher fiber content and lack pigmentation than Kabulis and hence a very low glycemic index which may make them suitable for people with high blood sugar problems. Kabuli Genotype show higher tolerance to salinity (Rao et al., 1995; Soussci et al., 1998). The present study compares Kabuli and Desi genotypes of chickpea in terms of growth, physiological and biochemical markers of salt tolerance. It will be helpful to select of chickpea varieties with improved salt tolerance for sustaining food production.

Materials and Methods

To assess the effect of saline water irrigation on chickpea, a field experiment was conducted in year 2007-2008. Plots of 1x1 m\textsuperscript{2} area were prepared and separated from each other by polythene sheets of 0.2 mm thickness, up to depth of 30 cm to stop leaching of salts between two adjacent plots. Plant to plant distance was 15 cm and plots were separated by a distance of 45 cm. The experiment was laid out in randomized block design with six replicates. Tubewell water of 1 dsm\textsuperscript{-1} EC was used to irrigate control sets. Saline solutions of 4, 8, 12, and 16 EC were prepared by mixing the salts NaCl, Na\textsubscript{2}SO\textsubscript{4}, NaHCO\textsubscript{3} and CaCl\textsubscript{2}. Three selected varieties PUSA-1053 (tolerant Kabuli), PUSA-939 (moderately sensitive Kabuli) and BG-256 (sensitive Desi) were grown in the experimental plots. Study the effect of
saline water irrigation on plant growth, nodulation, and leghemoglobin content at different growth stages. Saline water of 4, 8, 12 and 16 dsm\(^{-1}\) EC used to irrigate the plants at 20, 50 and 70 days after sowing (DAS). First sample of plants were collected at 30 DAS. Plant height, root and shoot length were recorded at 30 DAS. Root, shoot and leaves were dried in hot oven at 60°C for 48 hours and then dry weight of each were recorded. Same exercise was repeated at 60 and 90 DAS.

Relative growth rate (RGR) calculated as:

\[
RGR = \frac{(DM_2 - DM_1)}{t_2 - t_1} \text{ (g-g x d}^{-1})
\]

Where \(DM_1\) = initial total (shoot + root) dry mass, \(DM_2\) = final total dry mass, and \((t_2 - t_1)\) = time difference between two samplings (30 d).

At each stage number of nodules were counted and separated from the root. Detached nodules were then dried in hot oven at 60°C for 48 hours and dry weight was recorded. Afterwards this dried material was used for estimation of leghemoglobin.

### Table 1

| Variety | EC of Water dsm\(^{-1}\) | DWR (mg) | DWS (mg) | DM1 | DM2 | DM2-DM1 | RGR |
|---------|-----------------------------|----------|----------|-----|-----|---------|-----|
|         |                             | 30DAS    | 60DAS    | 30DAS | 60DAS |         |     |
| PUSA-1053 | 0                           | 63.1 ±4.33 | 75 ±5.22 | 430 ±4.25 | 535 ±4.33 | 493.1   | 610 | 116.9 | 3507 |
| 4       |                             | 60 ±3.32 | 65.6 ±3.43 | 300 ±3.27 | 350 ±2.45 | 360    | 415 | 55.6 | 1168 |
| 8       |                             | 55 ±4.33 | 58 ±2.25 | 275 ±3.25 | 320 ±4.53 | 330    | 378 | 48   | 1440 |
| 12      |                             | 40 ±4.21 | 50 ±2.45 | 210 ±4.33 | 240 ±3.54 | 250    | 290 | 40   | 1200 |
| 16      |                             | 35 ±3.35 | 45 ±3.25 | 195 ±4.53 | 220 ±3.35 | 230    | 265 | 45   | 1050 |
| PUSA-939 | 0                           | 52 ±3.26 | 72 ±3.54 | 300 ±4.37 | 400 ±3.28 | 372    | 472 | 100  | 3000 |
| 4       |                             | 50 ±4.25 | 60 ±3.53 | 260 ±4.33 | 310 ±3.27 | 316    | 370 | 54   | 1620 |
| 8       |                             | 35 ±3.35 | 40 ±3.43 | 240 ±3.26 | 280 ±4.21 | 275    | 320 | 45   | 1350 |
| 12      |                             | 27 ±5.55 | 30 ±3.44 | 180 ±3.28 | 215 ±3.27 | 207    | 245 | 38   | 1190 |
| 16      |                             | 27 ±3.27 | 35 ±3.28 | 130 ±3.26 | 160 ±5.55 | 157    | 195 | 32   | 960  |
| BG-256  | 0                           | 50 ±3.33 | 68 ±3.13 | 72 ±3.54 | 320 ±3.38 | 308    | 388 | 80   | 2400 |
| 4       |                             | 36 ±3.44 | 40 ±3.38 | 60 ±3.53 | 278 ±3.26 | 278    | 318 | 40   | 1200 |
| 8       |                             | 28 ±3.67 | 45.6 ±4.25 | 180 ±2.25 | 280 ±3.25 | 208    | 325.6 | 35 | 1050 |
| 12      |                             | 20.2 ±3.74 | 38.7 ±3.26 | 150 ±3.38 | 250 ±3.53 | 170.2  | 288.7 | 30 | 900  |
| 16      |                             | 18.3 ±3.77 | 25.1 ±4.47 | 100 ±3.33 | 220 ±4.25 | 118.3  | 245.1 | 20 | 600  |
there was about 50% reduction in shoot growth at 16 EC as a response of chickpea varieties to salinity and we found that to the reduction of nutrients transport from soil to the growing BG-256 (Desi). The decline in root dry mass is probably due reduction was seen in PUSA-1053 (Kabuli) and the largest in (Figure 1).

**Results and Discussion**

Salinity retards the plant growth from germination to maturity. This retardation is due to an increase in the osmotic pressure of the root medium and/or by the specific ion effects or the combination of both. The effect of salinity on plant height is shown in (Figure 1).

Effect of salinity on relative growth rate summarized with the help of dry weight of shoot and dry weight of root, there is a slight modification. Leghaemoglobin contents are expressed as mg heamin/gm fresh weight of nodules.

| Variety | EC of Water (dsm-1) | Days after sowing |
|---------|---------------------|------------------|
|         | 30 DAS | 60 DAS | 90 DAS |
| PUSA-1053 Control | 37±(3.27) | 93±(3.53) | 58±(4.25) |
| 4       | 47±(2.25) | 76±(4.25) | 45±(3.44) |
| 8       | 38±(4.25) | 64±(4.21) | 43±(3.35) |
| 12      | 20±(3.26) | 58±(3.13) | 35±(3.43) |
| 16      | 11±(4.25) | 47±(3.74) | 24±(3.13) |
| PUSA-939 Control | 54±(3.25) | 91±(4.21) | 72±(3.53) |
| 4       | 43±(3.54) | 74±(4.67) | 40±(2.53) |
| 8       | 34±(3.45) | 60±(4.56) | 35±(3.54) |
| 12      | 16±(4.54) | 55±(3.53) | 27±(4.21) |
| 16      | 8±(5.13)  | 45±(3.52) | 20±(3.58) |
| BG-256 Control | 50±(4.53) | 88±(2.21) | 62±(2.25) |
| 4       | 48±(3.53) | 70±(2.26) | 30±(2.26) |
| 8       | 30±(4.24) | 54±(2.21) | 25±(4.33) |
| 12      | 14±(3.21) | 47±(3.27) | 21±(3.46) |
| 16      | 4±(4.34)  | 38±(4.21) | 11±(2.27) |

Table 2. Effect of salinity on leghaemoglobin content of nodules in Cicer arietinum L. at 30, 60 and 90 DAS (data are expressed in mg/gm fresh wt. of nodules).

content of the nodules. For the sake of convenience and accuracy all the above observations were taken in six replicates and each replica carried ten plants. For the estimation of leghaemoglobin nodules were picked up at 30, 60 and 90 DAS. Nodules were carefully picked up from the roots and thoroughly washed with prechilled double distilled water. After washing nodules, blotted on the filter paper, weighed and finally crushed in prechilled sterilized mortar pestle containing 50 mM HCl, 5mM MgCl2, 20 mM KCI, 5 mM- mercapto ethanol. The slurry was centrifuged at 40C at 8,000 xg for 15 minutes. The pellets were discarded of legheamoglobin, also observed by Siddiqui et al. (1985) in barley, mung bean, chick pea and soybean (Garg, 2004; Mudgal, 2004; Rao et al., 2002; Zurayk et al., 1998). In Kabuli tolerant variety (PUSA-1053) shoot appear to be more sensitive to salinity than roots even at low salinity levels but in sensitive variety PUSA-939 and BG-256 roots are more sensitive. Cultivar BG-256 was adversely affected at all stages as compared to PUSA-1053 and PUSA-939.

The effect of salinity on the number and dry weight of nodules/plant at three durations (30, 60, 90 DAS) at 0, 4, 8, 12 and 16 dsm -1 salinity levels are shown in (Figure 2) and (Figure 3), respectively. Salinity delayed the onset of nitrogen fixation and prevented nitrogen fixation from resulting in significant growth but did not eliminate nodulations or the supply of nitrogen from nodule to shoot. Subbaroa et al. (1990) also reported that of the 4 strains effective under control only 2 were effective under salinity. In variety PUSA-1053 at 60 DAS had 60 nodule/plant in control sets and the number decreased to 22 nodules/plant at 16 dsm-1. The weight of nodules/plant was significantly decreased by salinity 30 days after sowing PUSA-1053 shows (68%), PUSA-939 (70%) and BG-256 (73%) reduction in weight of nodules/plant at highest salinity level as compared to control.

The failure of nodule formation at high salinity might be at the 4 strains effective under control only 2 were effective under salinity. In variety PUSA-1053 at 60 DAS had 60 nodule/plant in control sets and the number decreased to 22 nodules/plant at 16 dsm-1. The weight of nodules/plant was significantly decreased by salinity 30 days after sowing PUSA-1053 shows (68%), PUSA-939 (70%) and BG-256 (73%) reduction in weight of nodules/plant at highest salinity level as compared to control.

The failure of nodule formation at high salinity might be attributed to shrinkage of root hairs; consequently the reduction in plant growth under conditions of high salinity could only be partly accounted by the reduction or failure in nodulation (Tu, 1981; Mudgal et al., 2009). Zahran (1999) also showed that NaCl affected infection and nodulation. Dry weight of nodules is affected less adversely than its number.

Effect of salinity on leghaemoglobin content of nodules in three varieties of chickpea at 30, 60 and 90 DAS is given in (Table 2). It is evident from the data that leghaemoglobin content of the nodules decreased when the levels of salinity increase, however varietal differences were evident. In all varieties maximum leghaemoglobin content was observed at 60 DAS, which decreased thereafter due to aging and irreversible oxidation of leghaemoglobin, also observed by Siddiqui et al. (1985) in
pea nodules. In the early intracellular infection stage characteristic nitrogenase and leghemoglobin are absent or inactive. In the mature stage nitrogen fixation activity and leghemoglobin concentration become high. Sheokand et al. (1995) observed that NaCl treatment of the plants accelerated nodule greening, accompanied by a concomitant decrease in leghemoglobin (Lb) content of the nodules. With 50 mM NaCl, increase in duration of the treatment from 7 to 14 days, the Lb content recovered by 15% (rising from 41% to 56% of the control). However, under NaCl 100 mM treatment Lb content declined further dropping from 27% to 18% of the control 14 days after treatment (DAT). Babber et al. (2000) reported that leghemoglobin content of control plants decreased by 50% at day 85 indicating senescence of nodules. This senescence was further accelerated by salt treatment after which the leghemoglobin content fell to negligible levels. The structural changes associated with salt stress were mainly reduction in size of the nodules, decreased meristematic zone, reduced number and degradation of symbiosomes, reduced intercellular spaces and deposition of electron dense material in the intercellular spaces in the cortex of nodules. Inter- cellular spaces and deposition of electron dense material in the intercellular spaces in the cortex of nodules.

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