

RESPONSES OF *GOSSYPIUM HIRSUTUM* L. VARIETIES / LINES TO NaCl SALINITY AT SEEDLING

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ABSTRACT - Responses of fifty varieties/lines of *Gossypium hirsutum* L. to three NaCl salinity levels i.e., control, 10 dS/m and 20 dS/m, were compared at seedling stage. The results revealed that increase in NaCl salinity, after 30 days growth, considerably affected the root and shoot lengths, but the effect was more pronounced on roots. Based upon absolute salt tolerance and relative salt tolerance, using root length and shoot length data, three varieties/lines i.e., NIAB 78, B 557 and MNH 522 were found to be salt tolerant, and by contrast Qalandri, MNH 147 and BP52NC63 were found to be salt sensitive. In order to confirm the selection of the tolerant and sensitive varieties/lines, it was considered to study substrate salt tolerance of the selected varieties, the uptake of Na⁺ and K⁺ in the leaves, and then K⁺/Na⁺ ratio. The estimates of broadsense heritability of root length was high, suggesting that improvement in salinity tolerance in *Gossypium hirsutum* L. is possible exploiting the existing variation through selection and breeding.

Key words : *Gossypium hirsutum* L.; NaCl salinity; Heritability; Relative and absolute.

INTRODUCTION

The problem of soil salinity is of frequent occurrence in arid and semi arid regions (Neumann 1995; Ashraf, 1994; Lin *et al.*, 1997, Khan *et al.*, 2001). Due to continuous use of low quality irrigation water for agriculture purpose in the country, about 5.7x10⁶ ha of arable land had been effected by salinity (Mujtaba *et al.*, 2003). This engineering approach had been effective to decrease the concentration of salts to a greater extreme, but due to escalating cost of labor and energy these had become uneconomical to the country. Another concept, 'the genetic approach' which appears to be more feasible and economical, is the development of crop cultivars suitable for the areas affected by salinity. This

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approach is cheaper, and has been emphasized by many research workers (Qureshi *et al.*, 1990; Azhar and Khan, 1997; Hollington, 1998; Shannon *et al.*, 1998; Rao and McNeilly, 1999; Khan *et al.*, 2003; Madidi *et al.*, 2004). To start a breeding programme on salinity tolerance there must be available a significant amount of genetic variability in the gene pool of cotton. The presence of variation in salt tolerance has been reported by many research workers in different crops, for example, in wheat (Akhtar *et al.*, 2003; Bhatti *et al.*, 2004; Ali *et al.*, 2007), maize (Rao and McNeilly, 1999; Khan *et al.*, 2003), sorghum (Azhar and McNeilly, 1987, 2000, 2001), sunflower (Bhutta *et al.*, 2004), barley (Czembor, 2000; Dakir *et al.*, 2002; Madidi *et al.*, 2004), rice (Ahmad *et al.*, 1990; Shannon *et al.*, 1998; Ahmad *et al.*, 2000; Lee *et al.*, 2003).

Cotton (*Gossypium hirsutum* L.) is an important cash crop grown in the area affected by salinity in the country. Previous studies of salinity tolerance in cotton are relatively few but these provide strong evidence that variation existed within the species (Ashraf and Ahmad, 1999; 2000a, b; Noor *et al.*, 2001; Saqib *et al.*, 2002; Bhatti and Azhar 2002; Khan *et al.*, 2004; Akhtar *et al.*, 2005; Ali *et al.*, 2005). Recently Bhatti *et al.* (2006) and Azhar *et al.* (2007) have suggested that different varieties of Upland cotton responded differently to NaCl salinity. The present investigation further reports the extent

of variation within the species for NaCl salinity, and also the estimates of high heritability of the character.

MATERIALS AND METHODS

In this experiment, 50 cotton entries were grown in polythene bags of measuring 14.2cm × 20.3cm filled with soil. The EC_e and pH of the soil was determined prior to seedling, and there were 2.41 and 8.6, respectively. The response of 50 lines was assessed to three NaCl salinity levels i.e., control, 10 and 20 dS/m, and thus 150 polythene bags in each repeat were arranged following completely randomized design with three replications.

The seeds of each line were sown in each bag under proper moisture condition. Anhydrous NaCl was dissolved in Hoagland nutrient solution and the desired level of NaCl salinity in the polythene bags was progressively measured using two doses for 10 dS/m, and four doses for 20 dS/m. The first dose was applied to all the treatment bags one week after germination. The young seedlings were allowed to grow for two weeks under NaCl salinity. Eight seedlings were measured for root length (cm) and shoot length (cm). Based upon the measurement of these two characters, the responses of all the entries to increasing NaCl salinity were compared on absolute basis (Dewey, 1960), and relative basis (Maas, 1986). Relative salt tolerance may be defined as % growth of a genotype in salinized condition and compared with that in control. Relative salt tolerance was computed according to the following formula:

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$$\text{Relative salt tolerance (Salt tolerance index)} = \frac{\text{Value of a character in NaCl}}{\text{Value of a character in control}} \times 100$$

Estimates of broad sense heritability (h^2_B) of the two characters assessed under 10 dS/m and 20 dS/m and in control were made following the formula given by Falconer and Mackey (1996). The calculations were based upon the variance between-genotypes and within-genotypes.

RESULTS AND DISCUSSION

Development of salinity tolerance in a plant species can easily be made if considerable amount of genetically based variability is available to a plant breeder. Significant mean squares obtained from analysis of variance of root and

shoot lengths of 50 varieties/lines provided evidence that variation in the material was present, and the varieties responded differently to increasing NaCl salinity (*Table 1*). Growth of shoot and root length measured in 10 dS/m and 20 dS/m were compared with that in control conditions (relative salt tolerance). From the germplasm assessed a sub sample of 15 genotypes, based upon differences in root and shoot lengths, was taken for detailed description of genotype comparisons. Absolute and relative salt tolerances of 15 cotton genotypes are given in *Table 2 and 3*.

Table 1 - Mean squares for absolute root and shoot length of 50 genotypes tested in three NaCl levels

| Source of variation | DF | Root length | Shoot length |
|---------------------|-----|-------------|--------------|
| Genotypes (G) | 49 | 3.20** | 14.29** |
| Salinity level (S) | 2 | 221.37** | 193.99** |
| G x S | 98 | 0.64** | 0.88** |
| Error | 300 | 0.09** | 0.90** |

Root lengths of 15 genotypes differed in control as well as salinities (*Table 2*). When means of root length in two salinities were compared, differing responses of the genotypes were revealed, for example, PB494, MNH554, MNH147, VH142, BP52NC63, and Qalandri with shortest root lengths i.e., 3.49, 3.13, 3.24 and 2.85 cm respectively were revealed to have affected the most, and thus appeared to be sensitive genotypes.

Comparison of means showed that root lengths of MNH522, NIAB824, NIAB78, and B557 were affected less due to salinity, and with 5.66, 5.41, 6.21, and 5.78 cm, appeared to be salt tolerant. Comparison based upon relative root length data (index of salt tolerance) provided further estimates of the salinity tolerance of genotypes (*Table 2*). Means of root lengths revealed that some of the genotypes were more tolerant than others even at 10 dS/m

NaCl salinity, and PB843, MNH554, VH144, S12, MNH522, NIAB824, NIAB78, and B557 with 83%-93% tolerance index were shown to be less affected by salinity.

Under increased salinity i.e., 20 dS/m, root length of these accessions were significantly reduced, but the differences were still discernible, and MNH522, NIAB824, NIAB78, and B557 with 76% to 80% growth, whilst MNH147, BP52NC63 and Qalandri with salt tolerance index of 36%,

39%, and 39%, seemed to be sensitive to NaCl salt. Means of relative salt tolerance under low and high salinities revealed that MNH522, NIAB78, and B557 with 84%, 84%, 82% and 86% tolerance index were found to be salt tolerant accessions, and MNH147, BP52NC63 and Qalandri, with mean value showing greater of 50%, 51%, and 51% respectively seem to be most sensitive to salinity.

Table 2 - Mean performance of 15 genotypes based upon root length measured in control and two salinity levels

| Genotypes | Root length | | | | |
|-----------|-------------------------|-------|-------------------------|--------|-------|
| | Absolute salt tolerance | | Relative salt tolerance | | |
| | control | Means | 10dS/m | 20dS/m | Means |
| PB494 | 5.95 | 3.49 | 68.90 | 48.57 | 58.73 |
| PB843 | 6.51 | 4.44 | 86.78 | 49.61 | 68.20 |
| PB899 | 7.15 | 4.43 | 75.66 | 48.27 | 61.98 |
| MNH554 | 5.25 | 3.74 | 83.80 | 58.85 | 71.33 |
| FH634 | 7.23 | 4.47 | 71.23 | 52.55 | 61.89 |
| MNH147 | 6.75 | 2.84 | 64.00 | 36.44 | 50.22 |
| VH142 | 5.38 | 3.84 | 75.09 | 67.84 | 71.46 |
| VH144 | 5.17 | 4.12 | 89.74 | 69.63 | 79.69 |
| S12 | 6.49 | 4.75 | 83.20 | 63.17 | 73.18 |
| MNH522 | 6.71 | 5.66 | 92.69 | 76.00 | 84.35 |
| BP52NC63 | 6.27 | 3.12 | 64.11 | 39.07 | 51.59 |
| NIAB824 | 6.39 | 5.41 | 88.57 | 80.75 | 84.66 |
| NIAB78 | 7.59 | 6.21 | 87.62 | 76.15 | 81.88 |
| B557 | 6.74 | 5.78 | 92.72 | 78.78 | 85.75 |
| Qalandri | 6.26 | 3.23 | 64.05 | 39.13 | 51.59 |

Similar to root lengths, shoot lengths of 15 genotypes also differed in control as well as salinities (Table 3). Shoot length of MNH 522 was the longest (13.19cm) followed by NIAB 78 (12.57cm) in control. When means of shoot length in stress were

compared, different responses of the genotypes were revealed. Shoot lengths of MNH 522, NIAB 78 and B 557 were not reduced much under NaCl stress, and measured having 12.06, 11.44 and 11.2 cm, respectively, revealed high salt

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tolerance, and by contrast shoot lengths of MNH 147, BP52NC63, and Qalandri were affected the most due to salinities, and measured 6.27, 5.13

and 5.49 cm respectively, and thus appeared to be sensitive to NaCl stress.

Table 3 - Relative salt tolerance of 15 genotypes based upon shoot length measured in control and two salinity levels

| Genotype | Shoot length | | | | |
|----------|-------------------------|-------|-------------------------|--------|-------|
| | Absolute salt tolerance | | Relative salt tolerance | | |
| | control | Means | 10dS/m | 20dS/m | Means |
| PB494 | 10.5 | 9.11 | 78.36 | 71.08 | 74.72 |
| PB843 | 10.68 | 9.04 | 89.14 | 80.24 | 84.69 |
| PB899 | 10.18 | 8.91 | 93.03 | 82.02 | 87.52 |
| MNH554 | 9.75 | 7.95 | 87.08 | 76.00 | 81.54 |
| FH634 | 9.72 | 8.26 | 88.68 | 81.37 | 85.03 |
| MNH147 | 10.13 | 5.48 | 63.27 | 45.01 | 54.15 |
| VH142 | 9.56 | 6.26 | 67.99 | 63.07 | 65.53 |
| VH144 | 9.42 | 7.12 | 77.38 | 73.77 | 75.58 |
| S12 | 10.51 | 9.24 | 93.05 | 82.77 | 87.91 |
| MNH522 | 13.19 | 12.06 | 93.03 | 89.84 | 91.43 |
| BP52NC63 | 9.56 | 5.12 | 62.97 | 44.24 | 53.60 |
| NIAB824 | 10.22 | 8.49 | 88.64 | 77.49 | 83.07 |
| NIAB78 | 12.57 | 11.43 | 96.34 | 85.60 | 90.47 |
| B557 | 12.33 | 11.15 | 93.02 | 87.92 | 90.47 |
| Qalandri | 11.01 | 6.27 | 61.39 | 52.49 | 56.94 |

Shoot lengths of 15 genotypes were also markedly decreased and genotype Qalandri with a tolerance index of 61% appeared to be more sensitive than the others at 10dS/m (Table 3). In contrast, S12, MNH522, NIAB78, and B557 had the greatest index of salt tolerance of 93%, 93%, 93%, 96% and 93% respectively. Although shoot lengths decreased markedly at 20 dS/m, pronounced differences between genotypes are still evident for example, PB843, PB899, FH634, S12, MNH522, NIAB 78 and B557 with 80% to 90% relative values appeared to be tolerant, and MNH147, BP52NC63, and

Qalandri showed marked decrease in shoot length. From overall assessment of the germplasm, MNH522, NIAB78, and B557 with means of 90%, 91%, and 90% respectively appeared to be most tolerant. In contrast MNH147, BP52NC63 and Qalandri with relative values of 54%, 53%, and 57% were affected greatly by the salinities, and thus may be called salt tolerant accession. Similar varied responses to NaCl salinity at seedling stage of *Gossypium hirsutum* L. had been examined by Ashraf and Ahmad, 2000a, Akhtar *et al.*, 2005, Khan *et al.*, 2001, Khan *et al.*, 2004, Bhatti

and Azhar, 2002 and Noor *et al.*, 2001. In salt tolerance studies similar to the one reported here, Ashraf and Ahmad, 2000a also examined varieties MNH147 and B557 for salinity tolerance, and reported MNH147 as salt sensitive and B557 as salt tolerant.

Thus with respect to the potential of these varieties, present observations agreed to the previous reports, and similar was case of NIAB78 who had been reported as salt tolerant (Khan *et al.*, 2004). In an other studies on salinity tolerance, Azhar *et al.*, 2004, measured the root length of BP52NC63 found it susceptible to salinity as compared with other cultivars/lines. Thus the present research information appeared to be in accord with the previous research work.

The estimates of broadsense heritability of shoot and root lengths measured in 10dS/m and 20dS/m salinity levels and control are given in Table 4. The estimated genetic

variances for the absolute shoot length were 0.46 and 0.56 respectively, and h^2_B were 0.85, and 0.89, respectively. The h^2_B estimate for shoot length in un-salinized condition was 0.72. The genetic variance for absolute root length were 2.04 and 2.2 in low and high salinity levels, and the estimates of h^2_B being 0.94, and 0.96, respectively (Table 4). In previous work on salinity tolerance on cotton similar but varying magnitude h^2_{BS} of these had been reported, for example, 89% (Noor *et al.*, 2001), 83% (Akhtar and Azhar, 2001), 85% (Bhatti and Azhar, 2002). The broad-sense heritability estimates in sorghum ranged from 38% -78% (Azhar and McNeilly, 1987; Ashraf, 1994). Similar magnitude estimates of h^2_{BS} had been reported in wheat (Ali *et al.*, 2002). Thus based upon these estimates it is possible to improve salinity tolerance in cotton using high selection pressure.

Table 4 - Components of variance and broad sense heritability (h^2_B) of NaCl tolerance in 50 *Gossypium hirsutum* L. Varieties for root and shoot lengths in control and two NaCl salinity levels at seedling stage

| Components | Absolute root length | | | Absolute shoot length | | |
|-----------------|----------------------|---------------------|---------------------|-----------------------|---------------------|---------------------|
| | control | 10dSm ⁻¹ | 20dSm ⁻¹ | control | 10dSm ⁻¹ | 20dSm ⁻¹ |
| Vg | 1.11 | 2.04 | 2.2 | 0.32 | 0.46 | 0.56 |
| Vp | 1.41 | 2.17 | 2.27 | 0.44 | 0.53 | 0.63 |
| $H^2_B = Vg/Vp$ | 0.79 | 0.94 | 0.96 | 0.72 | 0.85 | 0.89 |

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