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# Sodium and Proline Accumulation as Osmoregulators in Tolerance of Sugar Beet Genotypes to Salinity

H. Pakniyat and M. Armion
Department of Crop Production and Plant Breeding, College of Agriculture, Shiraz University, Shiraz, Iran

**Abstract:** Twenty eight sugar beet genotypes were analysed for their tolerance at 3 NaCl levels (0, 3000 and 6000 mg NaCl $kg^{-1}$ soil) and $Na^+$, $K^+$, $Na^+/K^+$ and free proline were measured from the leaf samples. Results showed that increasing salinity level caused an increase in $Na^+$, $Na^+/K^+$ and proline, but a decrease in $K^+$ content of leaf samples ($p \leq 0.01$). As compared to non-tolerant genotypes, tolerant ones accumulated more $Na^+$ and $Na^+/K^+$ and proline and less $K^+$. It seems that $Na^+$ and proline accumulation in shoots are effective mechanisms for osmotic pressure adjustment and plant tolerance to salinity, a mechanism commonly seen in sugar beet ancestors.

**Key words:** Sugar beet, genotypes, salinity tolerance, osmotic adjustment, proline

## INTRODUCTION

Salinity is a serious environmental constraint to crop production in many parts of the world and the development of crops with improved salt tolerance is proposed as part of solution to this problem (Zhu, 2001). Plants follow different behaviors to combat salinity. Detailed reviews about salt tolerance mechanisms in different species are presented by Ashraf (2004) and Sairam and Tyagi (2004).

Osmotic adjustment has undoubtedly gained considerable recognition as a significant and effective mechanism of salinity tolerance in crop plants. Osmoregulatory effects of proline, glycine betaine and ions on water balance and salt tolerance, have been shown in spinach (Martino *et al*., 2003), wheat (Abdel-Aziz and Reda, 2000), bean (Shabala *et al*., 2000), cowpea (Freitas *et al*., 2001), sugar beet (Ghoulam *et al*., 2002; Heuer *et al*., 1981) and a halophyte sea aster (Ueda *et al*., 2003).

Sugar beet (*Beta vulgaris* L., family; *Chenopodiaceae*), has halophytes as ancestors. Its tolerance threshold to salinity is high (7 dS $m^{-1}$) (Katerji *et al*., 1997). Low tolerance to salinity is observed during seed germination and seedling emergence, but there are variations between sugar beet genotypes at high salt stresses (Sadeghian *et al*., 2000; Ghoulam *et al*., 2002).

Members of *Chenopodiaceae* including sugar beet can combat salinity by having osmotic regulating mechanisms due to accumulation of $Na^+$ and $Cl^-$ in their vacuoles and cytoplasm (Subbarao *et al*., 2001; Ghoulam *et al*., 2002). Sugar beet genotypes absorb $Na^+$ and accumulate it in their leaf tissue for regulation and adaptation of its osmotic potential with soil (Flowers, 1988). This may be the reason for considering sugar beet as a tolerant crop.

Although considerable research has been devoted to quantify the salt tolerance of the crop, data are usually based on comparisons among only a few cultivars and survey on a wide range of genotypes and wild progenitors of cultivated species have not been examined or exploited at all. The purpose of this experiment is to study the effect of $Na^+$, $K^+$, $Na^+/K^+$ and proline accumulation in 28 sugar beet genotypes, comparison of their salt tolerance and determination of osmotic adjustment role of $Na^+$, $Na^+/K^+$ and proline in tolerant and non-tolerant genotypes.

## MATERIALS AND METHODS

Twenty eight sugar beet genotypes (Table 1) were compared at 3 salinity levels (0, 3000 and 6000 mg NaCl $kg^{-1}$ soil) for their $Na^+$, $K^+$, $Na^+/K^+$ and proline contents in a completely randomized design with 3 replications. The experiment was conducted in a glasshouse at Agricultural College, Shiraz University in Badjgah, Iran in 2002. These genotypes were random regarding their salinity tolerance and some are planted by local formers in different part of Iran. Genotypes No. 2, 7 and 12 are known as salt tolerant and genotypes No. 4, 6 and 24 are known as non-tolerant ones. These genotypes were used as check genotypes in this experiment.

**Corresponding Author:** H. Pakniyat, Department of Crop Production and Plant Breeding, College of Agriculture, Shiraz University, Shiraz, Iran Tel/Fax: 0098-0711-2286134

Table 1: Sugar beet genotypes used for their salt tolerance determination in the experiment

| No. | Genotype | Embryo | Agronomic type |
|---|---|---|---|
| 1 | BR1 | Multigerm | NZ† |
| 2 | 7233* | Multigerm | E |
| 3 | PP22 | Multigerm | E |
| 4 | ICI** | Multigerm | N |
| 5 | IC2 | Multigerm | N |
| 6 | IC3** | Multigerm | N |
| 7 | 8001* | Multigerm | Z |
| 8 | H5505 | Multigerm | E |
| 9 | PP36 | Multigerm | NE |
| 10 | 41RT | Multigerm | E |
| 11 | 7233P012Xmst | Multigerm | NE |
| 12 | 7233P0107Xmst* | Multigerm | NE |
| 13 | PP18 | Multigerm | N |
| 14 | 9597MONO | Monogerm | Z |
| 15 | PP8 | Multigerm | N |
| 16 | 9585 | Monogerm | Z |
| 17 | Polyrave | Multigerm | N |
| 18 | DEZ | Multigerm | NZ |
| 19 | PP23 | Multigerm | NZ |
| 20 | 5708 | Multigerm | E |
| 21 | Simin 2 | Monogerm | Z |
| 22 | PP3 | Multigerm | E |
| 23 | Tribel | Multigerm | E |
| 24 | Universe** | Monogerm | N |
| 25 | Wild beet | Multigerm | - |
| 26 | Fodder beet | Multigerm | - |
| 27 | Red beet | Multigerm | - |
| 28 | Salad beet | Monogerm | - |

*: Tolerant check genotypes, **: Non-tolerant check genotypes, †: N = Normal, Z = Zucker, E = Ernte

Ten germinated seed were planted in 5 kg pots filled with a sandy clay loam soil (25% clay, 24% silt and 51% sand, EC = 0.57 dS $m^{-1}$).

Salt stress treatments were applied 4 weeks after planting and at 4 leaves stage. Salt (NaCl) stock solution was added to appropriate pots in 3 stages to final concentration based on soil field capacity. Four weeks after applying salt treatments (EC = 10 and 15 dS $m^{-1}$ for 3000 and 6000 mg NaCl $kg^{-1}$ soil, respectively), leaf samples were collected for $Na^+$, $K^+$ and proline measurements.

**$Na^+$ and $K^+$ measurements:** Leaves were cut from soil surface and, dried at 65°C oven for 48 h and made into fine powder using mortar. Samples (0.5 g) were ashed by putting them into crucibles and placed in 500°C electric furnace, 5 mL of 2N HCl were added to ash samples and mixed with boiling distilled water and filtered by filter paper (Whatman No. 2) into 50 mL volumetric flasks. $Na^+$ and $K^+$ were measured using flame photometer and reported as mg $g^{-1}$ dry weight.

**Proline measurement:** 0.5 g of leaf powder were mixed thoroughly in 10 mL sulfosalicilic acid (3%) and filtered by filter paper (Whatman No. 2) and the proline content was measured by Bates method (Bates *et al*., 1973).

**Statistical analysis:** Data were analysed using MSTATC and SPSS softwares. Correlation coefficients for the measured characters were also determined (Table 3). Graphical representations and diagrams were made from EXCEL software.

## RESULTS AND DISCUSSION

**$Na^+$ content:** Increasing salinity levels, increased leaf $Na^+$ contents of the genotypes (Fig. 1a). But the genotypes differed significantly ($p<0.01$) and the mean $Na^+$ content for tolerant genotypes (2, 7 and 12) were higher than non-tolerant (4, 6 and 24) ones (Fig. 2b and Table 2). Genotypes No. 23, 21, 19, 16, 20 and 9 were not significantly different regarding their $Na^+$ content and they may be considered as salt tolerant genotypes. On the other hand, genotypes No. 25, 1, 18, 26, 3, 15, 28 and 13 which have $Na^+$ contents within the range of non-tolerant check genotypes, may be considered as non-tolerant ones (Table 2). This variation in mechanism of $Na^+$ uptake could be due to some multiple adaptation to toxic ions operating concurrently within a specific plant (Tester and Davenport, 2003; Carden *et al*., 2003).

This is typical response of *Chenopodiaceae* family (halophytes), in which plant regulates its tissues osmotic potential by $Na^+$ accumulation and combating with salinity

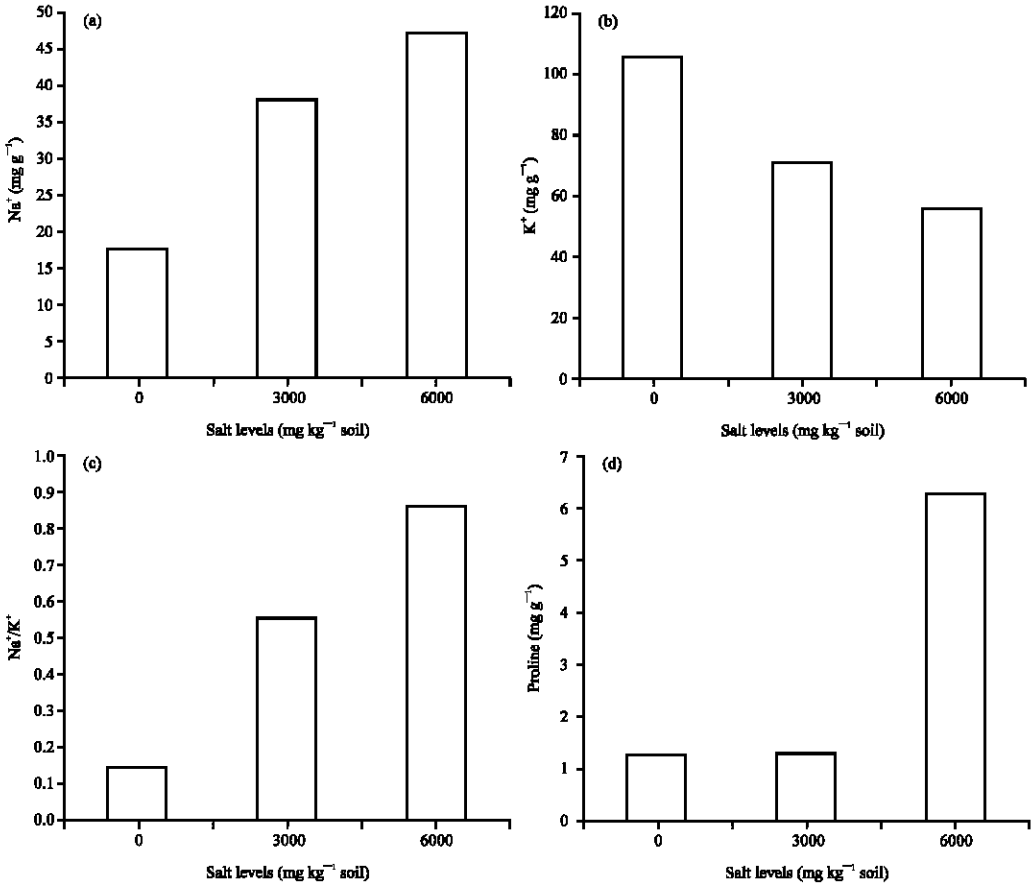


Fig. 1: Mean shoot $Na^+$, $K^+$, $Na^+/K^+$ and proline content of 28 sugar beet genotypes at 3 salt levels (0, 3000 and 6000 mg $kg^{-1}$ soil)

Table 2: Shoot $Na^+$, $K^+$, $Na^+/K^+$ and proline contents of 28 sugar beet genotypes under salinity (6000 mg NaCl $kg^{-1}$ soil) ranked on the basis of their $Na^+$ contents

| No. | $Na^+$ (mg $g^{-1}$) | $K^+$ (mg $g^{-1}$) | $Na^+/K^+$ ratio | Proline (mg $g^{-1}$) | No. | $Na^+$ (mg $g^{-1}$) | $K^+$ (mg $g^{-1}$) | $Na^+/K^+$ ratio | Proline (mg $g^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| 12* | 62.6a† | 54.4f | 1.15a | 12.27b | 5 | 46.0cd | 53.7f | 0.86c | 3.18h |
| 23 | 58.1a | 55.0e | 1.06a | 4.35f | 27 | 45.3d | 52.3f | 0.87c | 3.03hi |
| 21 | 55.0a | 66.3c | 0.83d | 7.35c | 17 | 45.0d | 46.0g | 0.99b | 3.81g |
| 19 | 53.0a | 47.0g | 1.13a | 2.98i | 4** | 44.0de | 42.0h | 0.68ef | 3.16h |
| 16 | 51.7a | 70.4b | 0.90bc | 3.16h | 13 | 43.3de | 71.7b | 0.60g | 5.32e |
| 7* | 51.3a | 56.7e | 1.05ab | 18.51a | 28 | 42.7ef | 32.3i | 1.40a | 6.57cd |
| 20 | 50.7a | 54.7ef | 0.92b | 5.00ef | 15 | 42.3f | 62.6d | 0.70e | 5.09e |
| 2* | 50.7a | 37.7hi | 1.35a | 17.84a | 3 | 42.3f | 42.0h | 1.00b | 7.38c |
| 9 | 50.0ab | 59.0d | 0.85cd | 5.21e | 24** | 41.7f | 69.7d | 0.59g | 3.74gh |
| 22 | 48.3b | 67.2c | 0.72e | 5.33e | 26 | 41.4f | 75.5a | 0.55g | 3.48h |
| 10 | 48.0bc | 51.7f | 0.93b | 6.36cd | 18 | 41.0fg | 65.3c | 0.63f | 3.88g |
| 8 | 47.7c | 74.5a | 0.64f | 3.88g | 1 | 40.0g | 47.3g | 0.85c | 4.12g |
| 14 | 46.7c | 53.0f | 0.88c | 4.85f | 25 | 39.7g | 66.7c | 0.59g | 6.03d |
| 11 | 46.0cd | 74.3a | 0.62f | 9.14c | 6** | 33.0h | 47.0g | 0.70e | 2.50i |

*: Tolerant check genotypes, **: Non-tolerant check genotypes, †: Means followed by the same letter(s) (a-i) in each column are not significantly different (DMRT, $p<0.01$)

(Eisa and Ali, 2001). This reaction is opposite to barley (a glycophyte crop) which is $Na^+$ excludant and in which salt tolerant genotypes accumulate less $Na^+$ in their shoots (Pakniyat *et al.*, 1997, 2003). It seems that in tolerant sugar beet genotypes simultaneous expression of proteinous vector of tonoplast membrane ($H^+$-ATPase port and $Na^+/H^+$ antiport) in cell vacoule of the leaves of tolerant genotypes are more than

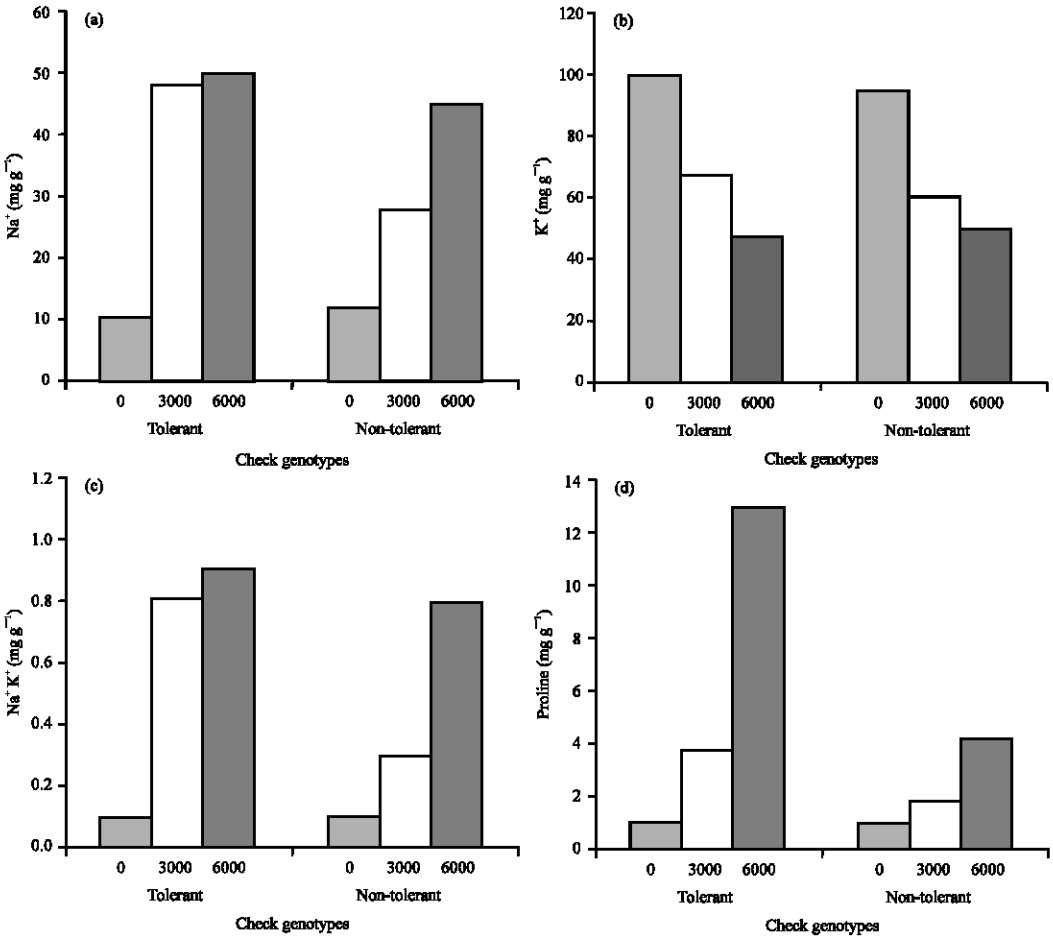


Fig. 2: Variation in mean $Na^+$, $K^+$, $Na^+/K^+$ and proline contents between tolerant (2, 7 and 12) and non-tolerant (4, 6 and 24) check sugar beet genotypes at different salinity levels (0, 3000 and 6000 mg $kg^{-1}$ soil)

Table 3: Correlation coefficients between $Na^+$, $K^+$, $Na^+/K^+$ and proline contents of sugar beet genotypes under salinity stress

| Parameters | $Na^+$ | $K^+$ | $Na^+/K^+$ | Proline |
|---|---|---|---|---|
| $Na^+$ | 1.00 | | | |
| $K^+$ | -0.85** | 1.00 | | |
| $Na^+/K^+$ | 0.90** | -0.94** | 1.00 | |
| Proline | 0.74** | 0.59** | 0.72** | 1.00 |

**: Significant at 1% probability level

non-tolerant ones (Kirsch *et al*., 1996; Shi *et al*., 2002; Parks *et al*., 2002).

**$K^+$ content:** Results showed that by increasing salinity level, mean $K^+$ content of the leaves of the genotypes were decreased and there were significant differences between salinity levels ($p<0.01$) in this regard (Fig. 1b). $K^+$ content was least for the third level of salinity (6000 mg $kg^{-1}$) and mean $K^+$ content for non-tolerant genotypes (2, 4 and 6) were higher than tolerant ones (2, 7 and 12) (Fig. 2b and Table 2). These trends have been observed in previous studies also (Flowers, 1985; Warne *et al*., 1990). This may be due to $Na^+$ role in regulation of sugar beet leaf osmotic potential (Lindhauer *et al*., 1990) and substitution of $Na^+$ with $K^+$ in this regard.

Negative correlation coefficient between leaf $Na^+$ and $K^+$ content was high and highly significant ($R = -0.85$, $p<0.01$) (Table 3). This agrees with the findings of Eisa and Ali (2001) results, which showed a negative linear correlation between these two ions after salt stress in sugar beet leaves. Increasing $Na^+$ accumulation and reduction of $K^+$ content shows a critical role of $Na^+$ in osmotic potential adjustment of sugar beet under salt stress.

**Leaf $Na^+/K^+$:** Results indicated a significant $Na^+/K^+$ ratio in leaves ($p<0.01$) of the genotypes with increasing salinity levels (Fig. 1c). Significant differences ($p<0.01$) were observed between sugar beet genotypes for their leaves $Na^+/K^+$ contents (Table 2). This ratio was higher in

tolerant genotype (2, 7 and 12) compared to non-tolerant ones (4, 6 and 24) (Fig. 2c). Genotypes 2, 12 and 28 had the highest leaf $Na^+/K^+$ ratios, of which genotypes No. 2 and 12 were among the check, known tolerant genotypes to salinity.

**Proline content:** Data on proline measurements indicated that proline accumulation has been the consequence of salinity and different salinity levels had significant effects ($p<0.01$) on leaf proline content of sugar beet genotypes. It seems that plant response to proline drastically increases at 6000 mg NaCl/kg soil (Fig. 1d). Genotypes were significantly different ($p<0.01$) regarding their leaf proline content under salinity (Table 2). Comparing proline content of tolerant and non-tolerant genotypes, it was indicated that tolerant genotypes accumulated higher level of proline than non-tolerant ones (Fig. 2d). This is in conformity with Gzick (1996) who concluded that higher proline level under salt stress is related to osmotic potential regulation in sugar beet.

In general salt tolerant sugar beet genotypes combat $Na^+$ toxicity by its accumulation in leaf cell vacuoles and regulate their osmotic potential under salinity stress. Besides, these genotypes accumulate higher level of proline in their leaf for their osmotic potential regulation. These findings are in agreement with those investigated in Atriplex which is a halophyte and belongs to *Chenopodiaceae* family (Glenn *et al*., 1994).

**Correlation coefficients:** Correlation coefficients between measured characters are shown in Table 3. There was positive and significant correlations between sugar beet shoot $Na^+$ and proline content ($r = 0.74$, $p<0.01$) (Table 3). These 2 measurements can be used for screening tolerant genotypes under salt stress.

Correlation between $Na^+$ and $K^+$ was negatively significant ($r = -0.85$, $p<0.01$) which shows osmoregulation of $Na^+$ ion under saline condition in tolerant genotypes and its substitution with $K^+$ (Table 3).

## CONCLUSIONS

The most tolerant genotype in this experiment was No. 12, which is among the tolerant check genotypes and the most non-tolerant genotypes was No. 6 which is among the non-tolerant check genotype. These two genotypes had the most and the least amount of $Na^+$ contents.

Regarding proline content, these genotypes were among the group genotypes which contained the highest and the lowest amount of proline content, respectively. Other tolerant and non-tolerant check genotypes followed the same trend regarding their $Na^+$ and proline content. These evidences confirm the osmoregulation role of $Na^+$ and proline in osmotic potential adjustment of the plant and hence its salt tolerance.

We may have crosses between genotypes 12 and 6 to obtain a segregating population to study quantitative trait loci involved in salt tolerance in the future.

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