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# THE ACCUMULATION AND COMPARTMENTATION OF PROLINE IN RELATION TO SALT TOLERANCE OF THREE SORGHUM CULTIVARS

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## SUMMARY

**Interactive effect of salinity stress and IAA on growth, water content (WC) and some relevant metabolic activities of three sorghum cultivars (45- days old) were studied. Dry matter (DW), water content and tolerance index (TI) of the tested sorghum cvs. differed in response to salinity. Cvs. Dorado and Hagen Shandawil tolerated salinity up to the level of 4 and 2 bar NaCl, respectively, while cv. Giza 113 did not show tolerance to salinity stress. This was accompanied with differences in accumulation of carbohydrate and nitrogen compounds. Proline accumulation seems to be in response to injury. It was positively correlated with the growth criteria in cv. Dorado (the most resistance cultivar) and to some extent in cv. Hagen Shandawil, while negatively correlated in cv. Giza 113 (the most sensitive cultivar). Salinity stress increased markedly the protein content in the salt sensitive cultivar (Giza 113), which was accompanied with a drastic reduction in growth and pigmentation. IAA ameliorated the inhibitory effect of salinity on the growth, increased carbohydrates and protein content of all the three cultivars. IAA, also markedly retarded the accumulation of proline in most cases. The relationship between salt tolerance of sorghum cultivars and the changes in proline content is discussed.**

***Key words*** **: Amino acids, carbohydrate, indole acetic acid, protein, tolerance index, water content.**

## INTRODUCTION

Differences in salt tolerance exist not only among different genera and species, but also within the same species. For example, there are reports on the response to salinity of different varieties of barley (Flowers and Hajibagheri 2001) and wheat (Azooz 2002, Ismail 2003). Comparing the response of cultivars of the same species to salinity provides a convenient and useful tool for unveiling the basic mechanisms involved in salt tolerance.

The reduction in plant growth is a consequence of several physiological responses including modification of water status, photosynthetic efficiency, carbon allocation and utilization (Nabil and Coudret 1995).

Protection from osmotic stress injury is accomplished by the accumulation of organic osmolytes. Carbohydrates accumulate in various plants under salinity condition (Balibrea *et al*. 1997). Salt stress is known to inhibit protein synthesis in various plant tissues (Evers *et al*. 1997) due to the inhibition of amino acids incorporation into protein. Protein hydrolysis in salinized plants is always associated with increase in proline and free amino acids (Irigoyen *et al*. 1992).

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Although the precise role of proline accumulation is still debated, proline is often considered to act as a compatible solute involved in osmotic adjustment. The accumulation of proline may be through an increase in its synthesis concomitantly with inhibition of its catabolism (Yoshiba *et al*. 1997). Changes in proline levels in several crops and cell cultures have been correlated with their ability to tolerate or adapt to salinity (Chowdhury *et al*. 1993). However, its role in imparting resistance to salt - stress is controversial.

The major effect of salinity in the roots environment has been attributed to reduced hormone delivery from root to leaves, which could induce an inhibition of crop growth. Hence, various growth-promating substances such as $GA_3$, IAA or kinetin have been used to overcome the drastic effect of salt stress (Singh *et al.* 1994, Aldesuquy *et al.* 1998).

The purpose of this study was to compare the tolerance of three sorghum cultivars viz. Dorado, Hagen Shandawil and Giza 113 to salinity stress, and their behaviour in respect to osmosolutes and proline accumulation. Exogenous application of IAA was done in order to assess whether the effect of salinity stress on dry matter, water content, tolerance index and some relevant metabolic activities (pigments, carbohydrates, proteins, total free amino acids and proline) might be alleviated. The tested cultivars were chosen according to preliminary experiments on the growth of some sorghum cultivars under saline conditions.

## MATERIALS AND METHODS

The seeds of sorghum cultivars were obtained from the breeding program of Agricultural Research Center, Dokky, Cairo, Egypt.

Seeds of sorghum cultivars (cvs. Dorado, Hagen Shandawil and Giza 113) were sown in weighed plastic pots (10 seeds/pot) containing dry clay soil. The pots were irrigated with water and left until emergence of seedlings. Thereafter, the pots were watered to the salinization levels: 0 (control), 1, 2, 3 ,4 and 5 bar. After three days from irrigation with NaCl solution, plants shoots were sprayed with an aqueous solution (25 ppm) of IAA. After a week of spraying, the plants were irrigated with second dosage of salinity: 0 (control), 1, 2, 3 ,4 and 5 bar. After 3 days from second dosage of NaCl, plants were sprayed again with an aqueous solution (25 ppm) of IAA. Plants were kept in growth chamber maintained at 32 / 28 °C day/night (12 h) temperature cycles, and light intensity of 105 mol $m^{-2}s^{-1}$. The plants were irrigated daily with water to reach the above desired salinization levels.

After 30 days of sowing, the photosynthetic pigments in leaves were determined as described by Lichtenthaler and Wellburn (1983). At the end of the experimental period (45 days), the dry matter of roots and shoots was determined after drying the freshly harvested organs (roots and shoots) in an aerated oven at 80°C to constant weight. Tolerance index (TI) was calculated according to De Le Rosa- Ibarra and Maiti (1995). Carbohydrates were determined by the anthrone sulphuric acid method (Fales 1951, Schlegel 1956) and adopted by Badour (1959). Proteins were determined according to Bradford (1976). Proline was extracted by 5- sulfosalicylic acid and determined according to Bates *et al*. (1973). Free amino acids were estimated according to the method of Lee and Takahashi (1966).

Each treatment was replicated thrice and the data of all experiments were subjected to analysis by the least significant differences test (L.S.D) using SPSS program.

## RESULTS AND DISCUSSION

Dry matter of root and shoot, water content, tolerance index and pigment contents of the three sorghum cultivars differed in response to salinity stress (Table 1). Cv. Dorado and Hagen Shandawil tolerated salinity up to the level of 4 and 2 bar NaCl respectively, while cv. Giza 113 was sensitive to salinity stress. This inhibitory effect of salinity on dry matter and water content was more pronounced in cv. Giza 113 than cv. Hagen Shandawil and Dorado, especially at the higher salinization levels. However, there were significant differences among the three sorghum cultivars at the highest salinity concentration (10 bar NaCl) in the reduction of dry matter yield and consequently the tolerance index of root (about 0.63, 0.45 and 0.36) and shoot (about 0.51, 0.32 and 0.22) of cv. Dorado, Hagen Shandawil and Giza 113, respectively, as compared with control (no NaCl). The low and moderate salinity levels induced insignificant decrease in chl.a, chl.b

**Table 1.** Effect of salinity and treatment with IAA on dry matter (g plant$^{-1}$), water content %, tolerance index (TI) and pigment contents (mg g$^{-1}$ fresh weight) of three sorghum cvs. (cvs. Dorado, Hagen Shandawil and Giza 113).

| Treatments | NaCl (bars) | Root | | | Shoot | | | Pigments | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | DW | %WC | TI | DW | %WC | TI | Chl. a | Chl. b | Carot. |
| **cv. Dorado** | | | | | | | | | | |
| NaCl | 0 | 0.340 | 87.5 | 1.00 | 0.899 | 83.3 | 1.00 | 3.42 | 1.81 | 1.66 |
| (Ref. | 2 | 0.327 | 87.3 | 0.96 | 0.860 | 83.6 | 0. 95 | 3.09 | 1.56 | 1.57 |
| Control) | 4 | 0.319 | 86.9 | 0.94 | 0.836 | 82.9 | 0.93 | 2.90 | 1.30 | 1.43 |
| | 6 | 0.287 | 85.3** | 0.84 | 0.776 | 80.3** | 0.86* | 2.17** | 1.32 | 1.32 |
| | 8 | 0.245 | 84.2** | 0.72** | 0.598** | 78.6** | 0.67** | 1.11** | 0.67** | 0.69** |
| | 10 | 0.215* | 80.5** | 0.63** | 0.456** | 80.0** | 0.51** | 1.01** | 0.64** | 0.55** |
| NaCl+ | 0 | 0.386 | 89.3** | 1.14 | 1.099* | 84.8** | 1.22** | 4.05 | 2.60* | 1.95 |
| 25 ppm | 2 | 0.355 | 89.0** | 1.04 | 0.988 | 85.2** | 1.10 | 4.78** | 2.60* | 2.36 |
| IAA | 4 | 0.335 | 88.5* * | 0.99 | 0.864 | 84.8** | 0.96 | 4.23* | 2.64* | 2.22 |
| | 6 | 0.281 | 88.3** | 0.82* | 0.797 | 83.6 | 0.89 | 2.51* | 1.40 | 1.49 |
| | 8 | 0.227* | 88.6** | 0.67** | 0.660** | 83.3 | 0.73** | 1.62** | 0.93* | 0.96 |
| | 10 | 0.216* | 87.5 | 0.64** | 0.586** | 81.1** | 0.65** | 1.39** | 0.89* | 0.80* |
| L.S.D. | 5% | 0.111 | 0.7 | 0.17 | 0.157 | 0.9 | 0.16 | 0.797 | 0.70 | 0.709 |
| | 1% | 0.149 | 0.8 | 0.23 | 0.212 | 1.2 | 0.22 | 1.073 | 0.95 | 0.955 |
| **cv. Hagen Shandawil** | | | | | | | | | | |
| NaCl | 0 | 0.433 | 85.6 | 1.00 | 0.873 | 83.1 | 1.00 | 3.174 | 2.188 | 1.849 |
| (Ref. | 2 | 0.425 | 85.5 | 0.98 | 0.844 | 83.3 | 0.96 | 3.339 | 1.470** | 1.611 |
| Control) | 4 | 0.327 | 83.0** | 0.76** | 0.737 | 81.2** | 0.90 | 3.495 | 1.628** | 1.740 |
| | 6 | 0.306 | 78.4** | 0.71** | 0.662** | 80.4** | 0.75** | 2.595 | 1.361** | 1.660 |
| | 8 | 0.283 | 76.2** | 0.65** | 0.449** | 80.0** | 0.57** | 0.625** | 0.351** | 0.387** |
| | 10 | 0.195* | 75.1** | 0.45** | 0.313** | 79.0** | 0.32** | 0.600** | 0.320** | 0.340** |
| NaCl+ | 0 | 0.686* | 85.1 | 1.58** | 1.041* | 84.3** | 1.07 | 5.790** | 2.892** | 2.571 |
| 25 ppm | 2 | 0.608 | 86.0 | 1.40** | 0.941 | 84.6** | 1.08 | 4.061** | 2.057 | 1.751 |
| IAA | 4 | 0.555 | 82.4** | 1.28** | 0.811 | 84.1** | 0.98 | 4.048** | 2.105 | 1.589 |
| | 6 | 0.538 | 82.8** | 1.24** | 0.683* | 82.9 | 0.78** | 4.449** | 2.297 | 1.765 |
| | 8 | 0.366 | 80.0** | 0.85 | 0.453** | 82.6 | 0.52** | 2.009** | 0.986** | 0.784* |
| | 10 | 0.300 | 76.1** | 0.69** | 0.398** | 81.2** | 0.41** | 2.642 | 0.965** | 0.986* |
| L.S.D. | 5% | 0.223 | 0.8 | 0.16 | 0.148** | 0.8 | 0.15 | 0.582 | 0.397 | 0.808 |
| | 1% | 0.300 | 1.1 | 0.20 | 0.199 | 1.1 | 0.21 | 0.785 | 0.535 | 1.088 |
| **cv. Giza 113** | | | | | | | | | | |
| NaCl | 0 | 0.500 | 74.5 | 1.00 | 0.890 | 81.8 | 1.00 | 2.710 | 1.672 | 0.701 |
| (Ref. | 2 | 0.448 | 74.1 | 0.90 | 0.744 | 78.0** | 0.84 | 2.638 | 1.263** | 0.684 |
| Control) | 4 | 0.341* | 68.6** | 0.68** | 0.716 | 75.0** | 0.80* | 1.472** | 0.663** | 0.368** |
| | 6 | 0.331* | 67.5** | 0.66** | 0.654 | 73.0** | 0.73** | 1.52** | 0.696** | 0.406** |
| | 8 | 0.219** | 66.2** | 0.44** | 0.262** | 70.1** | 0.29** | 0.788** | 0.373** | 0.188** |
| | 10 | 0.180** | 64.3** | 0.36** | 0.192** | 69.6** | 0.22** | 0.928** | 0.528** | 0.278** |
| NaCl+ | 0 | 0.602 | 76.1* | 1.20* | 0.956 | 82.3 | 1.07. | 3.381 | 2.756** | 0.771 |
| 25 ppm | 2 | 0.511 | 82.0** | 1.02 | 0.846 | 80.7* | 0.95. | 3.333 | 2.145** | 0.731 |
| IAA | 4 | 0.484 | 77.4** | 0.97 | 0.808 | 78.7** | 0.91 | 3.321 | 1.439** | 0.861* |
| | 6 | 0.451 | 76.7** | 0.90 | 0.740 | 77.8** | 0.83 | 2.683 | 1.39** | 0.666 |
| | 8 | 0.393 | 75.6 | 0.77* | 0.292** | 76.0** | 0.33** | 2.032 | 0.490** | 0.52** |
| | 10 | 0.272** | 75.3 | 0.54** | 0.254** | 72.8** | 0.29** | 2.131 | 0.805** | 0.431** |
| L.S.D. | 5% | 0.137 | 1.3 | 0.19 | 0.255 | 1.0 | 0.18 | 0.734 | 0.136 | 0.128 |
| | 1% | 0.185 | 2.0 | 0.31 | 0.344 | 1.5 | 0.25 | 0.988 | 0.184 | 0.172 |

* Significant differences ($P$ = 0.05) and ** Highly significant differences ($P$ = 0.01) as compared with control (0.0 NaCl).

and carotenoids content in cv. Dorado, and chl.a and carotenoids only in cv. Hagen Shandawil. Significant decline in all the pigments was observed in cv. Giza 113, when compared with control unsalinized plants.

The reduction in growth could be attributed to reduction in cell division and/or in cell enlargement (Hopkins, 1999). Our results on dry matter yield and water content of the three sorghum cultivars (as responses to salinity stress), ranked them as follows, in order from most to least tolerant: Dorado>Hagen Shandawil>Giza 113. Consequently, tolerance index was higher in cv. Dorado than the other two sorghum cultivars. This has been related to the increase in succulence and water content. Thus, cv. Dorado had consistently higher RWC than Hagen Shandawil or Giza 113.

Spraying 25 ppm IAA alleviated the inhibitory effect of salinity on dry matter yields, water content, tolerance index and pigment contents of sorghum cultivars, and markedly increased the above mentioned parameters over those of the unsalinized plants, especially at the low and moderate salinity levels. There was positive response of spraying on several aspects (dry matter, water content, tolerance index and chl.b) even in control (zero salinity). This alleviation effect of IAA may be due to the increase in the green area, leading to a considerable increase in carbohydrates and proteins, which might play the major role in water status (conservation and utilization).

The effect of salinity on the contents of carbohydrate and protein (Table 2) varied. In the root system of sorghum cultivars, soluble carbohydrates at low and moderate salinity levels did not show significant differences between the control and salinity treatment, while there was a significant reduction at higher salinity level. On the other hand, the insoluble carbohydrates significantly increased with increasing salinity levels. In the shoot of both cv. Dorado and Hagen Shandawil, carbohydrates remained more or less unchanged up to the level of 6 bar NaCl, thereafter there was marked decline as compared with unsalinized plants. Protein contents (soluble and insoluble) increased significantly in the roots of cv. Hagen Shandawil and Giza 113, while in cv. Dorado the opposite trend was observed with increasing salinity. In the shoot system of both cvs. Dorado and Hagen Shandawil, protein content was considerably reduced except the soluble fraction in cv. Hagen Shandawil which accumulated with increasing salinity. In case of Giza 113, salinity stress exhibited almost no change in soluble (at all salinity levels) and insoluble proteins (up to the level of 6 bar NaCl), but insoluble protein decreased significantly at 8 and 10 bar salinity levels.

The decrease in soluble carbohydrates of sorghum cvs. under salinity stress was at the expense of increase in the insoluble fraction. This indicates that carbohydrates did not play any role in osmoregulation, and could be due to the transition of plant from a state of osmoregulation (survival) to a state of growth. In the shoots of sorghum cultivars, the pattern of changes in carbohydrates was similar to that of chlorophyll, which gives a reason to believe that low chlorophyll content causes a relevant reduction of light absorption by leaves (Evans, 1996), and consequently reduces the biosynthesis of carbohydrates.

The marked increase in protein contents of Hagen Shandawil and Giza 113 root (the most sensitive cultivars) was accompanied with a marked reduction in growth, of both cvs. Thus, under these conditions, it can be pointed out that the two cultivars divert most of the synthesised protein from a state of growth to a state of osmoregulation (survival), thus proteins might play the major contribution in osmotic adjustment in these two cultivars. In cv. Giza 113 and to some extent in cv. Hagen Shandawil the accumulation of proteins was accompanied with a marked reduction in photosynthetic pigments and consequently in carbohydrates. The salt sensitive cultivars, synthesizes the protein for survival rather than for growth. Sultana *et al.* (2002) found the same correlation between pigmentation and protein, and attributed such accumulation of protein at the expense of pigmentation to be used osmotically.

Spraying the salinized plants with IAA resulted generally, in higher carbohydrate and protein contents than in unsprayed plants. This is also true even at 0.00 bar NaCl. The contents of soluble proteins in root and shoot were higher in cv. Dorado when compared with Hagen Shandawil or Giza 113. The pronounced accumulation of carbohydrates and proteins due to IAA treatments, may be attributed to the obvious increase in green area, which consequently leads to an increase in photosynthetic activity and consequently in plant productivity and dry matter production.

**Table 2.** Effect of salinity and treatment with IAA on carbohydrate and protein, (soluble and insoluble) contents (mg $g^{-1}$ dry matter) of root and shoot of three sorghum cultivars (cv. Dorado, Hagen Shandawil and Giza 113).

| Treatments | NaCl (bars) | Carbohydrates | | | | Proteins | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Root | | Shoot | | Root | | Shoot | |
| | | Soluble | Insoluble | Soluble | Insoluble | Soluble | Insoluble | Soluble | Insoluble |
| **cv. Dorado** | | | | | | | | | |
| NaCl | 0 | 9.16 | 203.31 | 25.75 | 202.56 | 132.40 | 142.55 | 216.25 | 129.95 |
| ( Ref. | 2 | 7.64 | 200.92 | 29.01 | 185.98 | 138.15* | 151.65** | 215.85 | 119.85** |
| Control) | 4 | 7.96 | 209.49 | 24.75 | 188.25 | 124.65** | 150.30** | 210.90 | 118.10** |
| | 6 | 6.73* | 217.68** | 24.69 | 181.36* | 113.20** | 143.55 | 199.35** | 113.90** |
| | 8 | 6.65* | 231.67** | 19.59** | 153.60** | 90.95** | 125.70** | 177.95** | 99.75** |
| | 10 | 4.57** | 226.77** | 10.27** | 139.16** | 89.60** | 102.20** | 157.70** | 94.85** |
| NaCl+ | 0 | 13.64** | 221.78** | 31.99** | 208.33 | 134.10 | 151.65** | 213.85 | 126.35 |
| 25 ppm | 2 | 14.16** | 221.26** | 31.22* | 209.97 | 134.83 | 153.65** | 213.30 | 120.45** |
| IAA | 4 | 13.11** | 244.06** | 34.32** | 222.40* | 131.40 | 146.95 | 213.05 | 120.30** |
| | 6 | 10.87 | 227.53** | 29.28 | 201.52 | 128.05 | 153.65** | 222.95* | 119.80** |
| | 8 | 12.36* | 268.65** | 24.17 | 185.31 | 118.95** | 147.60* | 234.70** | 110.00** |
| | 10 | 8.19 | 237.05** | 15.86** | 149.81** | 141.50** | 141.55 | 222.90* | 125.65 |
| L.S.D. | 5% | 2.42 | 8.04 | 4.70 | 17.34 | 5.27 | 4.60 | 6.45 | 7.00 |
| | 1% | 3.26 | 10.82 | 5.49 | 23.33 | 7.10 | 6.15 | 8.70 | 9.40 |
| **cv. Hagen Shandawil** | | | | | | | | | |
| NaCl | 0 | 6.91 | 145.87 | 29.40 | 196.89 | 72.75 | 70.80 | 183.75 | 82.90 |
| (Ref. | 2 | 5.96 | 146.02 | 29.03 | 211.54 | 86.22* | 93.00** | 181.25 | 91.30* |
| Control) | 4 | 6.93 | 165.08* | 30.87 | 186.93 | 83.55 | 87.45** | 183.00 | 79.85 |
| | 6 | 6.18 | 180.36** | 30.02 | 178.43 | 80.85 | 81.90* | 198.15** | 81.20 |
| | 8 | 5.43 | 209.13** | 20.97** | 159.50** | 85.25* | 79.70 | 204.65** | 75.15* |
| | 10 | 3.94** | 220.45** | 14.86** | 126.20** | 87.40** | 77.50 | 211.15** | 76.30* |
| NaCl+ | 0 | 5.66 | 167.18* | 31.88 | 216.28* | 79.50 | 86.30** | 196.45** | 87.65 |
| 25 ppm | 2 | 17.58** | 151.08 | 32.36 | 217.35* | 89.60** | 98.75** | 201.50** | 106.60** |
| IAA | 4 | 8.20 | 186.39** | 38.21** | 197.644 | 81.19 | 90.95** | 206.05** | 98.10** |
| | 6 | 5.22 | 204.27** | 46.03** | 198.76 | 88.95** | 84.25* | 203.15** | 94.15** |
| | 8 | 7.15 | 280.71** | 33.26 | 164.08** | 74.10 | 78.55 | 220.32** | 87.95 |
| | 10 | 9.17* | 318.63** | 22.31** | 166.32** | 81.85 | 89.65** | 193.75* | 83.60 |
| L.S.D. | 5% | 2.17 | 16.57 | 4.14 | 18.97 | 10.75 | 10.65 | 7.55 | 5.70 |
| | 1% | 2.93 | 22.32 | 5.57 | 25.55 | 14.50 | 14.35 | 10.40 | 9.25 |
| **cv. Giza 113** | | | | | | | | | |
| NaCl | 0 | 5.96 | 192.50 | 46.93 | 178.05 | 68.4 | 117.95 | 134.10 | 80.85 |
| (Ref. | 2 | 5.66 | 224.09** | 41.19** | 178.72 | 70.6 | 130.25** | 148.70** | 84.05 |
| Control) | 4 | 4.92 | 255.68** | 28.64** | 175.31 | 72.75** | 142.55** | 132.25 | 79.85 |
| | 6 | 4.47 | 196.08 | 25.92** | 168.89 | 71.75* | 140.20** | 129.75** | 80.65 |
| | 8 | 3.42* | 129.48** | 25.70** | 163.52* | 70.75 | 133.45** | 133.60 | 72.45** |
| | 10 | 2.68** | 101.62** | 20.97** | 130.40** | 75.15** | 130.05** | 133.85 | 71.90** |
| NaCl+ | 0 | 7.69 | 223.55** | 55.24** | 208.33** | 81.85** | 134.80** | 151.95** | 92.50** |
| 25 ppm | 2 | 9.16** | 186.32 | 44.28** | 223.31** | 77.5** | 137.20** | 158.70** | 87.45** |
| IAA | 4 | 9.60** | 170.16** | 41.08** | 225.62** | 72.75** | 149.30** | 161.25** | 79.70 |
| | 6 | 8.90* | 226.48** | 33.74** | 245.32** | 81.85** | 151.65** | 170.00** | 79.00 |
| | 8 | 7.45 | 172.22** | 30.13** | 252.96** | 86.40** | 145.95** | 159.40** | 76.80* |
| | 10 | 5.96 | 109.06** | 30.17** | 242.50** | 90.95** | 135.85** | 163.45** | 75.15** |
| L.S.D. | 5% | 2.24 | 8.61 | 3.78 | 13.86 | 3.05 | 3.90 | 3.00 | 4.30 |
| | 1% | 3.02 | 11.60 | 5.09 | 18.94 | 4.10 | 5.30 | 4.05 | 4.55 |

* Significant differences ($P$ = 0.05) and ** Highly significant differences ($P$ = 0.01) as compared with control (0.0 NaCl)

Increasing salinity levels resulted in a marked accumulation of total free amino acids in root and shoot of both cv. Dorado and Giza 113 (Table 3). Salinity stress induced insignificant changes in shoot and a sharp reduction in root of total free amino acids of cv. Hagen Shandawil. The contents of free amino acids were higher in cv. Dorado, as compared with the two other cultivars.

Significantly increased proline accumulation was observed in the root system of cv. Hagen Shandawil and cv. Giza 113 only at high salinity levels (Table 4). In shoots of cv. Dorado, there was an accumulation of proline content with increasing NaCl salinity up to the level of 6 bar NaCl, above which it decreased smoothly, while in cv. Hagen Shandawil, salinity stress exhibited insignificant changes in proline content. In Giza 113, proline content increased markedly with increasing salinity level in the soil.

The accumulation and distribution of proline among the sorghum cvs. even at the control levels, seem to be complicated based on the following observations:

[1] The higher amounts of proline in cv. Dorado (the more salt tolerant) even in roots and shoots of absolute control than that in cv.Giza 113 (the least salt tolerant) indicate that proline shows close correlation with the phenotypic variation of the three sorghum cvs.

[2] There are two opposite situations in the criteria of proline, according to the type of cultivar, the plant organ as well as the salinity level used: it is positively correlated in cv. Dorado and to some extent in cv. Hagen Shandawil but negatively correlated in cv. Giza 113.

[3] However, if we take into consideration the relation between the absolute amount of proline and water content, the data reveal that the plant organ which accumulated higher proline, is the plant organ which had higher water content. These results let us to concluded that the positive correlation between proline and WC is probably due to the capacity of sorghum cultivars to reduce the water potential and change the osmotic gradient, assuring the water flow to the plant (De La Rosa-Ibarra and Maiti, 1995).

Spraying with IAA in most cases resulted in pronounced increase of total free amino acids in root (except in root of cv. Dorado) and shoot system of the three sorghum cvs. IAA markedly retarded the accumulation of proline in most cases except in shoot of cv. Hagen Shandawil, where a significant increase was observed as compared with the corresponding salinized plants. This means that proline is an injury symptom rather than a salt tolerance sensor (Lutts *et al.* 1996).

**Table 3.** Effect of salinity and treatment with IAA on total free amino acids content (mg $g^{-1}$ dry matter) of root and shoot of sorghum cultivars.

| Treatments | NaCl (bars) | cv.Dorado | | cv. Hagen Shandawil | | cv. Giza 113 | |
|---|---|---|---|---|---|---|---|
| | | Root | Shoot | Root | Shoot | Root | Shoot |
| NaCl | 0 | 6.812 | 9.251 | 2.436 | 12.912 | 0.864 | 6.704 |
| (Ref. | 2 | 7.231 | 19.606** | 2.096 | 14.796* | 2.515** | 7.752 |
| Control) | 4 | 8.279 | 29.259** | 1.048** | 12.955 | 3.039** | 9.409** |
| | 6 | 9.903** | 18.204** | 1.126** | 13.158 | 3.563** | 10.256** |
| | 8 | 11.528** | 18.807** | 1.126** | 14.324 | 3.353** | 10.408** |
| | 10 | 12.209** | 13.855** | 1.388** | 11.792 | 3.144** | 10.103** |
| NaCl+25 ppm IAA | 0 | 6.812 | 14.852** | 1.834* | 9.473** | 3.301** | 14.255** |
| | 2 | 7.493 | 22.503** | 1.310** | 8.428** | 3.93** | 10.607** |
| | 4 | 8.296 | 25.655** | 1.126** | 9.907** | 3.93** | 10.756** |
| | 6 | 6.104 | 21.656** | 1.388** | 13.185 | 3.301** | 12.005** |
| | 8 | 6.812 | 20.058** | 1.659* | 13.303 | 3.222** | 11.454** |
| | 10 | 6.969 | 25.504** | 2.960 | 11.386 | 3.301** | 11.002** |
| L.S.D. | 5% | 1.497 | 2.410 | 0.579 | 1.699 | 1.106 | 1.782 |
| | 1% | 2.016 | 3.246 | 0.780 | 2.289 | 1.490 | 2.400 |

* Significant differences ($P$ = 0.05) and ** Highly significant differences ($P$ = 0.01) as compared with control ( 0.0 NaCl)

**Table 4.** Effect of salinity and treatment with IAA on proline content (mg $g^{-1}$ dry matter) of root and shoot of sorghum cultivars.

| Treatments | NaCl (bars) | cv.Dorado | | cv. Hagen Shandawil | | cv. Giza 113 | |
|---|---|---|---|---|---|---|---|
| | | Root | Shoot | Root | Shoot | Root | Shoot |
| NaCl (Ref. | 0 | 4.892 | 3.352 | 1.881 | 1.334 | 0.408 | 0.486 |
| Control) | 2 | 4.512 | 4.346* | 1.724* | 1.192 | 0.479 | 0.814** |
| | 4 | 4.750 | 5.104** | 1.567** | 1.360 | 0.551 | 1.092** |
| | 6 | 4.868 | 4.358* | 1.900 | 1.249 | 0.726** | 1.272** |
| | 8 | 4.987 | 2.883 | 2.299** | 1.541 | 0.741** | 1.470** |
| | 10 | 4.512 | 2.519* | 3.610** | 1.456 | 0.807** | 1.574** |
| NaCl+ | 0 | 2.042** | 1.803** | 0.456** | 1.480 | 0.251* | 0.308 |
| 25 ppm IAA | 2 | 1.570** | 4.694** | 0.522** | 1.256 | 0.251* | 0.351 |
| | 4 | 1.140** | 3.926 | 0.536** | 1.880** | 0.427 | 0.394 |
| | 6 | 0.475** | 3.077 | 0.313** | 3.403** | 0.536 | 0.957** |
| | 8 | 0.427** | 1.795** | 0.499** | 3.094** | 0.408 | 1.002** |
| | 10 | 0.332** | 1.484** | 0.518** | 3.197** | 0.489 | 1.234** |
| L.S.D. | 5% | 0.855 | 0.794 | 0.127 | 0.214 | 0.152 | 0.292 |
| | 1% | 1.152 | 1.070 | 0.172 | 0.289 | 0.205 | 0.393 |

* Significant differences ($P$ = 0.05) and ** Highly significant differences ($P$ = 0.01) as compared with control (0.0 NaCl)

From a quantitative point of view, it can be concluded that sorghum cultivars differ in their strategy towards the accumulation of proline under salinity conditions and /or even under the interactive effect of salinity and IAA treatments.

In addition, the increase in free amino acids in cv. Dorado was associated with a decrease in protein, while increase in soluble protein and proline in cv. Hagen Shandawil were associated with a decrease of amino acids content. On the other hand, the increase in soluble proteins, total free amino acids and proline in cv. Giza 113, were not associated with a decrease in the other nitrogen compounds (may result from the decrease in chlorophyll content). This means that the strategy of osmoregulation was different among the tested sorghum cultivars.

Accordingly, it can be concluded that the three sorghum cultivars differing in their strategy towards the accumulation of soluble solutes under salinity conditions and/or even under the interactive effect of salinity and IAA treatments.

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