# IMPACT OF PROLINE ON PHYSIOLOGICAL PARAMETERS UNDER SODICITY IN SUNFLOWER (*HELIANTHUS ANNUUS*)

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**Abstract**

A study of changes in physiological condition of different sunflower genotypes in sodic soil was attempted. Sunflower genotypes were grown under salt stress conditions in a glasshouse. Proline, relative water content and chlorophyll content were estimated in plants grown in both normal and sodic soil. A reduction in relative water content and chlorophyll content and a significant increase in proline content were observed under stressed conditions. The decrement up to the tune of 16.80 and 55.87 per cent was evidenced under stress for RWC and total chlorophyll content, respectively. The chlorophyll content was reduced as a correlated response of decrease in RWC. Proline as an osmoregulant and its compensatory mechanism towards reduction in RWC and photosynthetic pigment was established. The difference in genetic potential towards salt tolerance among sunflower inbreds was realized. The inbreds SF7 and 400B were identified as salt enduring genotypes.

## Introduction

Soil salinity is one of the major environmental stresses and it adversely affects plant growth and metabolism. It poses serious limitations to agriculture in many areas around the world, particularly on irrigated farmlands. Plant breeding has confirmed that salt tolerance is not conferred by a single trait, but is the consequence of complex gene interactions (Lutts et al., 1996). As a result, progress in understanding the network of molecular mechanisms leading to salt tolerance has been slow (Rausch et al., 1996). The dogmatic view is that some of the factors assumed to limit plant growth in salt stress are turgor, photosynthesis and production of particular metabolic products (Taneja et al., 1992; Al-Zahari and Hajar, 1998). At this juncture, exploitation of genetic variation towards salt tolerance is the better way to combat such complexity. Selection of all the traits attributed to salt endurance that become apparent only during stress is difficult. Hence, efforts to identify the basic adaptive trait which triggers the homeostatic nature of the genotype to the salt stress become indispensable. Proline has been found to be the osmoregulant to maintain turgor under salt stress (Heur, 1999; Kogen et al., 2000) However, studies showing the compensation effect on chlorophyll content and relative water content is limited. The present paper aims at finding out the impact of increased production of proline on RWC and chlorophyll content in salt stress situations and its reflection in increased yield under stress conditions.

## Materials and Methods

To study the sodicity of sunflower genotypes and the impact of salt towards physiological parameters, a total of twenty-five inbreds were grown under glasshouse conditions. The investigation was carried out at Tamil Nadu Agricultural University, Coimbatore during Rabi (winter) 2002. Earthen pots with a capacity of eight kilograms of soil were used. Two conditions, i.e., control and stress were maintained. For the control, soil from normal cultivated land (sandy loam) was used, and for stress, the soil was transported from a sodic tract of Tamil Nadu (Tiruchirappalli). The soil was utilized as such and no additional fertilizer was applied. Five seeds from each genotype were sown for each treatment. Ten days after sowing, three plants were maintained in each pot. The pots were drenched with water daily up to the soil saturation point. The setup was replicated twice and the treatments were randomized within the replication. EC of the soil (dS/m) at the time of sampling; control: 1.59, and stress: 1.76. The pH of the soil at the time of sampling was 7.08 for the control and 8.80 for the stressed. EC of the irrigation water was 1.03 dS/m.

The leaves were collected at star bud stage of anthesis of the crop as it is considered to be critical for stress condition at which maximum reduction in seed filling is reported (Prabudeva et al., 1998). Proline content of the leaves (2-4 leaves from the top) was estimated by the method described by Bates et al., (1973) and expressed as μg/g of fresh weight. Relative water content (RWC) was estimated 2nd, 3rd, or 4th leaf from the top of the plant by method of Weatherly (1950) and was expressed in percentage. Chlorophyll a and b were estimated in the fully expanded third leaf from the top by the method of Yoshida et al. (1972) and the contents were expressed as mg/g of fresh weight. The data were analyzed statistically for significance using the "Agristat 2000" computer package.

## Results and Discussion

Significant variation in proline accumulation existed among genotypes, between treatments and in the interactions between genotype and treatment. Proline content ranged from 97.25 to 389.9 μg/g dry weight (Table1). Stressed plants manifested a general increase in proline accumulation to the tune of 1.5 fold compared to plants grown under normal conditions. This observation is in accordance with the reports of Santos et al. (1999), Navarai-Izzo et al. (1992) and Kogen et al. (2000) in sunflower. Such an increase could be due to the stimulation of proline synthesis from glutamate by the loss of feedback inhibition, the decline in proline oxidation or the decrease of its incorporation into protein (Kramer, 1983). The percent increase of proline was greater in tolerant than in susceptible genotypes. This may enable the former genotypes to cope with the salt stress in a comparatively efficient manner (Singh and Singh, 1999), because proline can serve as a protector of enzyme denaturation, a reservoir of nitrogen and carbon or a stabilizer of the machinery for protein synthesis (Hamada and Khulaef, 1995). Following these criteria, the genotypes SF7, SF34, SF83, SF60, GP336, GP324 and 400B are grouped under tolerant and the genotypes Morden, 302B, GP161, SF45, 336B and SF91 are classified as susceptible. The yield pattern shows a perceptible reduction in yield of plants under stress compared to potential yield. Though the

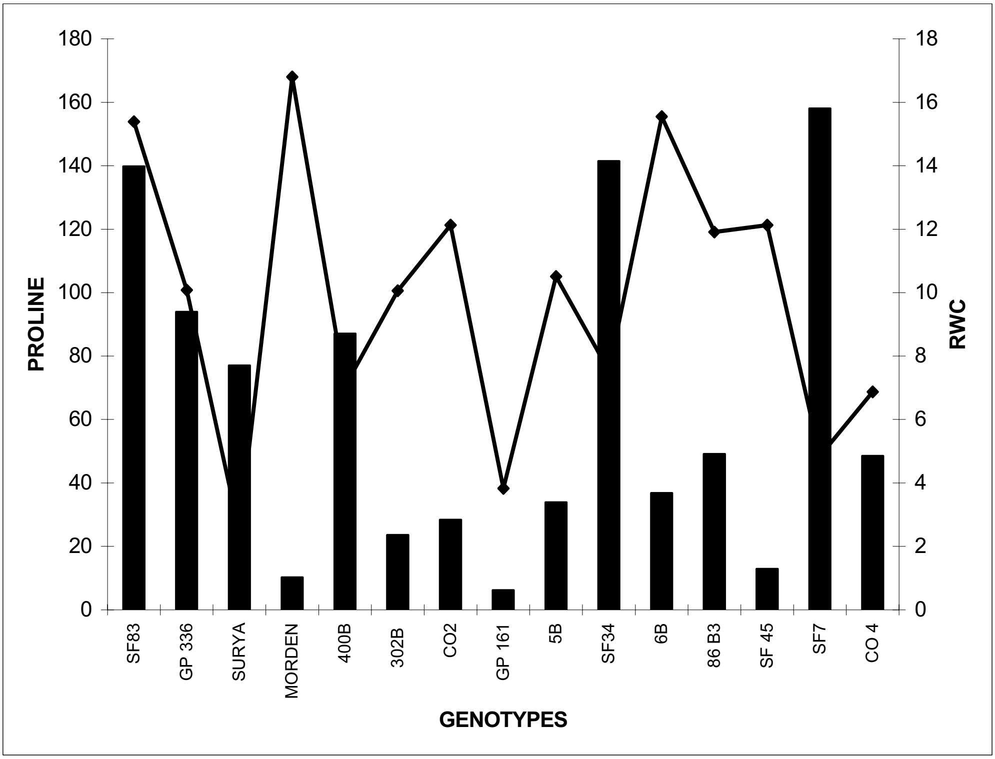


Figure 1. Comparison of RWC with proline content.

yield potential depends on genetic potential of the inbreds, the realization of the potential yield under stress varies with the endurance capacity of the inbreds. In this study, the genotypes which had been grouped as tolerant based on proline accumulation had shown lower reduction in seed yield (Table 1) and vice versa. This forms evidence that proline reduces the salt induced reduction to certain extent.

Reduction of moisture under stress has been well established (Taneja et al., 1992; Djanaguiraman, 2000). The results of the present investigation also showed a decrease to the tune of 9.11 percent compared to the control (Table 1), and a significant variation was observed among the genotypes, between treatments and their interaction with genotypes. Even a moderate reduction in leaf water potential severely affects leaf area index to about 33 percent in stressed conditions compared to normal (Sorrentino et al., 2000). Genotypes studied showed a reduction in range of 2.47 percent to 16.80 percent in RWC under stressed conditions. Based on proportionate per cent decrease of RWC in the genotypes under stressed conditions, the genotypes are grouped as tolerant and susceptible because even a unity decrease in water potential will lead to higher saturation point, which reduces the turgor pressure drastically. This ultimately results in reduced growth and yield of the plants (Blum, 1974). Accordingly, Morden, 6B, SF83, SF45, CO2, 86B3, GP336 and GP336 are grouped under susceptible, since they manifested a higher percent decrease in RWC than the genotypes studied, whereas Surya, GP93, GP161, SF7, SF54 and CO4 are grouped as tolerant.

Table 1. Sunflower genotypes and selected traits.

| Genotypes | Proline content (ug/g of dry weight) | | | Relative water content (%) | | | Total chlorophyll content (mg/g) | | | Single plant Seed yield (g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Stress | Per cent increase | Control | Stress | Per cent difference | Control | Stress | Per cent decrease | Control | Stress | Per cent Decrease |
| CO 2 | 263.50 | 338.40 | 28.43 | 88.86 | 78.08 | 12.13 | 2.046 | 1.252 | 38.81 | 30.02 | 19.58 | 34.78 |
| MORDEN | 389.90 | 429.90 | 10.26 | 96.65 | 80.41 | 16.80 | 1.702 | 0.751 | 55.87 | 27.10 | 20.15 | 25.65 |
| CO 4 | 179.40 | 266.50 | 48.55 | 92.08 | 85.75 | 6.87 | 1.324 | 0.937 | 29.23 | 30.66 | 20.59 | 32.84 |
| SURYA | 110.50 | 195.63 | 77.04 | 91.01 | 88.76 | 2.47 | 1.535 | 1.365 | 11.07 | 26.01 | 21.92 | 15.72 |
| CO 3 | 317.20 | 416.98 | 31.46 | 90.30 | 81.35 | 9.91 | 1.457 | 1.022 | 29.85 | 31.51 | 19.59 | 37.83 |
| 302 B | 162.20 | 200.45 | 23.62 | 94.64 | 85.12 | 10.06 | 1.397 | 1.352 | 3.22 | 23.56 | 13.59 | 42.32 |
| 6B | 252.10 | 345.00 | 36.85 | 96.03 | 81.10 | 15.55 | 2.084 | 1.351 | 35.17 | 27.28 | 19.59 | 28.19 |
| 400 B | 105.96 | 198.25 | 87.10 | 92.02 | 85.53 | 7.05 | 1.343 | 1.311 | 2.38 | 25.75 | 15.46 | 39.96 |
| 86B3 | 130.83 | 195.10 | 49.12 | 92.70 | 81.66 | 11.91 | 1.463 | 1.157 | 20.91 | 24.46 | 22.89 | 6.42 |
| 5B | 130.65 | 174.95 | 33.91 | 92.76 | 83.01 | 10.51 | 1.073 | 0.854 | 20.41 | 22.34 | 18.95 | 15.17 |
| 336B | 247.10 | 315.65 | 27.74 | 90.29 | 82.33 | 8.82 | 2.210 | 1.687 | 23.66 | 21.92 | 14.98 | 31.66 |
| GP324 | 131.95 | 246.29 | 86.65 | 92.95 | 84.22 | 9.39 | 1.495 | 1.331 | 10.97 | 28.59 | 21.12 | 26.13 |
| GP93 | 230.63 | 349.21 | 51.42 | 90.55 | 87.32 | 3.57 | 1.419 | 1.074 | 24.31 | 35.40 | 23.41 | 33.87 |
| GP86 | 241.15 | 286.59 | 128.84 | 95.05 | 83.79 | 11.85 | 1.948 | 1.399 | 28.18 | 35.04 | 21.17 | 39.58 |
| GP336 | 119.60 | 231.95 | 93.94 | 92.60 | 83.27 | 10.08 | 1.767 | 1.390 | 21.33 | 33.57 | 29.89 | 10.96 |
| GP161 | 186.85 | 198.51 | 6.24 | 86.08 | 82.78 | 3.83 | 1.764 | 1.454 | 17.57 | 39.79 | 33.35 | 16.18 |
| GP255 | 150.15 | 261.30 | 74.03 | 93.50 | 84.79 | 9.32 | 1.486 | 1.039 | 30.08 | 25.40 | 15.00 | 40.94 |
| SF83 | 130.65 | 313.30 | 139.80 | 95.27 | 84.38 | 15.39 | 0.983 | 0.654 | 33.46 | 17.91 | 15.30 | 14.57 |
| SF45 | 254.15 | 285.13 | 12.19 | 91.74 | 80.61 | 12.13 | 1.059 | 0.930 | 12.18 | 26.46 | 19.61 | 25.89 |
| SF34 | 104.00 | 251.12 | 141.46 | 88.70 | 82.01 | 7.54 | 1.292 | 0.929 | 28.09 | 24.84 | 16.74 | 32.61 |
| SF30 | 111.80 | 148.85 | 33.14 | 88.97 | 84.04 | 5.54 | 1.342 | 1.213 | 6.61 | 24.64 | 20.56 | 16.56 |
| SF60 | 105.95 | 252.85 | 138.65 | 91.42 | 82.47 | 9.79 | 1.136 | 1.106 | 2.64 | 29.91 | 27.44 | 8.26 |
| SF7 | 97.50 | 251.65 | 158.10 | 93.16 | 88.64 | 4.85 | 2.057 | 1.983 | 3.59 | 32.56 | 30.56 | 6.14 |
| SF91 | 142.35 | 185.62 | 30.40 | 91.74 | 85.78 | 6.50 | 1.211 | 1.143 | 5.61 | 28.16 | 22.64 | 19.60 |
| SF54 | 102.35 | 136.16 | 33.03 | 93.82 | 88.38 | 5.80 | 1.607 | 1.192 | 25.82 | 26.89 | 24.49 | 8.93 |
| MEAN | 175.93 | 259.01 | 58.88 | 92.12 | 83.82 | 9.11 | 1.528 | 1.195 | 23.30 | 27.99 | 21.14 | 24.43 |
| | SE (d) | CD (0.05) | CD (0.01) | SE (d) | CD(0.05) | CD (0.01) | SE (d) | CD (0.05) | CD (0.01) | SE (d) | CD (0.05) | CD (0.01) |
| Genotype | 6.40 | 12.86 | 17.152 | 4.197 | 8.43 | 11.247 | 0.167 | 0.335 | 0.447 | 0.710 | 1.426 | 1.903 |
| Treatment | 1.81 | 3.634 | 4.851 | 1.18 | 2.38 | 3.181 | 0.047 | 0.095 | 0.126 | 0.200 | 0.404 | 0.538 |
| G x T | 9.05 | 18.183 | 24.256 | 5.935 | 11.923 | 15.905 | 0.236 | 0.047 | 0.632 | 1.00 | 2.018 | 2.691 |

However, the genotypes which have been grouped under susceptible based on relative decrease in water content are not actually susceptible towards salt stress, because the decrease in RWC by salt stress is well compensated by an increase in osmotic potential through an increased accumulation of amino acids, viz., proline, total free amino acids and nutrients (Taneja et al., 1992). As a result, the pressure potential is maintained. This study also evidenced a similar trend. As can be seen from the graph (Figure 1) for the genotypes SF83, GP336, 400B and SF34, the decline in RWC is well compensated by the increased proline accumulation. The difference of yield between normal and stress conditions in the aforementioned genotypes is much less and it is concluded that proline's compensatory mechanism to RWC stabilised the yield. Hence these genotypes, though termed as susceptible based on the RWC parameter, are considered as tolerant genotypes, whereas the genotypes Morden, CO2, 302B, 5B, 6B, 86B3 and SF45 are confirmed as susceptible, as these genotypes suffered due to high percent reduction of RWC under stress coupled with low proline accumulation. Perusal of the yield pattern in normal and sodic soils indicate a significant reduction in seed yield in the genotypes that are conferred susceptible by the RWC status of the plant (Table1).

Reduction in plant turgor as a correlated response results in stomatal closure leading to reduction in intercellular CO2 partial pressure (Downton et al., 1985). Hence a reduced photosynthetic rate ultimately manifests low yield under stress. As increased proline accumulation maintains osmoticum under stress and maintains the stomatal activity, the photosynthetic rate under salt stress in high proline accumulators even with reduced water content in leaves can maintain the normal photosynthetic rate in relation to yield is stability. Observation of yield patterns in normal and sodic soils also indicated significant reduction in seed yield in the genotypes that are identified susceptible by both RWC and proline parameter whereas the genotypes which had high proline accumulation during stress manifested a meager reduction in seed yield compared to the potential (normal) yield (Table1).

Total chlorophyll content decreased in stressed plants. The decrease in chlorophyll content was mainly attributed to the destruction of chlorophyll a, which is more sensitive to salt stress than chlorophyll b (Reddy and Vora, 1986), the increased activity of chlorophyll degrading enzyme chlorophyllase (Rao and Rao, 1981) and ion accumulation in leaves and flowers (Yeo, 1983). Some genotypes expressed a lower reduction in chlorophyll content while certain genotypes exhibited drastic reduction. The decrease ranged from 2.64 to 55.87 percent. This observation concurs with reports of Prasad and Srivastava (1991) and Al-Zaharani and Hajar (1998). In this investigation, the genotypes which had high accumulation of proline showed less reduction in total chlorophyll content, which may be due to the ionic balance attributed to proline accumulation. Moreover, the closure of stomatal cells during sodicity-induced drought stress reduces the availability of CO2 for photosynthesis, which can lead to the formation of reactive oxygen species from the misdirection of electrons in the photosystem (Ajay Arora et al., 2002) especially singlet oxygen and free radicals which are known to break down DNA (Wei et al., 1998). Such increased production of toxic O2 species under severe stress has been shown to increase susceptibility to photo inhibition with subsequent development of chlorosis (Wise and Naylor, 1987). The singlet oxygen (1O2) is reported to have direct involvement in photo bleaching of photosynthetic pigments (Mishra et al., 1994). Proline with its chemical properties has been shown to protect plants against singlet oxygen and free radical induced damages (Matysik et al., 2002; Alia et al., 1994) Hence the total chlorophyll content may be stabilized in high proline accumulating plants. In this context, the stability of total chlorophyll content might produce high photosynthetic efficiency and eventually high yield (Peiris and Ranasinghe, 1993), regarded as the index

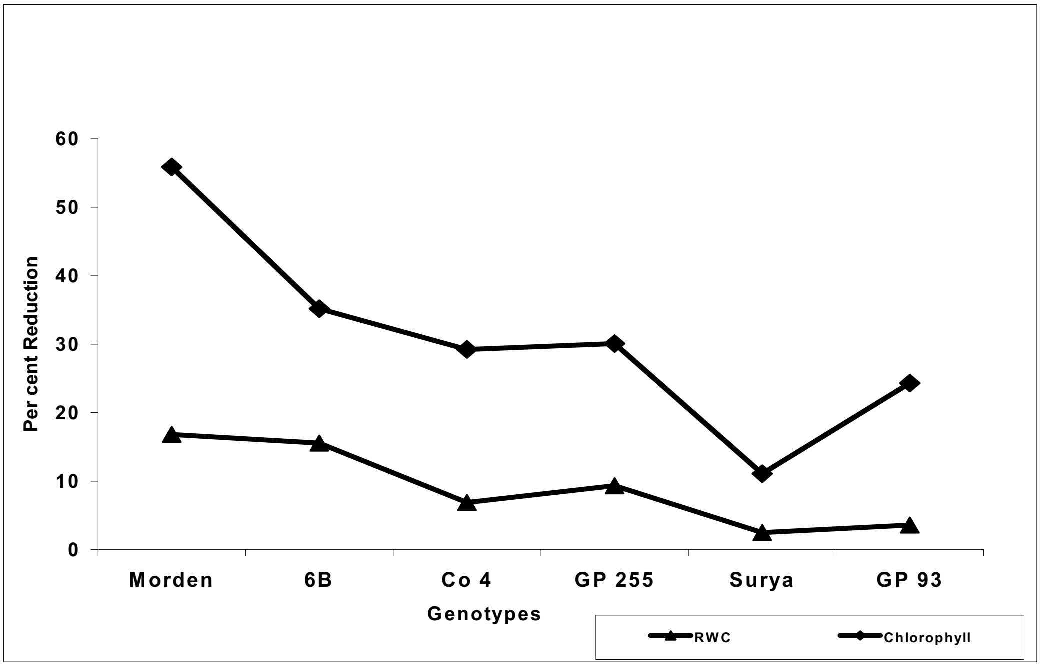


Figure 2. Response of RWC and chlorophyll content to salt stress.

of tolerance. Based on this, the genotypes 400B, SF60, SF7, SF91, Surya, GP324 and SF45 are grouped as tolerant. The genotypes which showed maximum reduction, viz., Morden, CO2, 6B, SF83 and GP255 are grouped as susceptible.

Thus the established fact is that the reduction in the total chlorophyll content of salt-stressed genotypes compared to normal is attributed to the decrease in available water under salt-affected conditions of soil (Valia et al., 1993). Contradicting this, Nieves et al. (1991) reported that the decrease in chlorophyll content in plants under salt stress is due to increased water content (succulence) especially in the case of chloride salinity. To identify the cause for reduction in chlorophyll content in this experiment the RWC and total chlorophyll contents obtained in this study were compared. For this, the genotypes which were classified as tolerant, moderately tolerant, and susceptible based on RWC have been considered (Figure 2). A positive correlation between percent reduction in RWC and total chlorophyll content was established and formed a point of substantiation to the reports of Valia et al., (1993) and Garcia et al. (1992).

## Conclusions

From this experiment, it can be concluded that proline is triggered when plants are exposed to salt stress and acts as a temporary osmoticum (Pessarakkali, 1999). It acts as a compensatory mechanism for reduction in leaf water potential and chlorophyll content. Hence, it can be considered as an essential screening index for salt tolerance. The sunflower inbreds SF7 and 400B were accordingly identified as tolerant to salt stress as they passed all the three screening indices.

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