

BREEDING SUNFLOWER FOR SALT TOLERANCE: PHYSIOLOGICAL BASIS FOR SALT TOLERANCE IN SUNFLOWER (*Helianthus annuus* L.)

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ABSTRACT:

Fifteen sunflower genotypes were studied for ion accumulation in leaves. A high degree of genetic variability was found among sunflower genotypes for accumulation of ions. Chloride ions accumulation increased at moderate and high salinity. Sodium ions accumulation decreased at moderate salinity but increased at higher level of salinity while potassium ions accumulation first increased at moderate salinity then decreased at higher salinity level. A similar behaviour was observed for K^+/Na^+ ratio in young leaves at moderate and high salinity levels. Sunflower genotypes showed tolerance to salinity by maintaining Na^+ at lower and K^+/Na^+ ratio at higher level in young growing leaves and it was found to be a good criterion of salt tolerance in sunflower.

Key words: *Helianthus annuus* L., ion accumulation, salinity tolerance.

INTRODUCTION

Soil salinity is a serious hazard to agriculture in the arid, semi-arid and irrigated areas of the world. Pakistan lies in the similar region, therefore, salt accumulation is one of the major factors limiting the proper utilization of land for agricultural purposes in the country. About 80 percent of the salt affected soils of the Punjab and 56 percent of Pakistan are saline-sodic and are not reclaimable because of a low permeability of canal water (Muhammed, 1983). In general, $NaCl$, Na_2SO_4 , $MgCl_2$, and $MgSO_4$ dominate in saline soils, while the sodic soils mainly contain Na_2CO_3 and $NaHCO_3$ salts (Szabolcs, 1980). Ionic ratios are quite variable in salt affected soils of different regions. Na^+ dominates among cations while anions Cl^- and SO_4^{2-} are equally distributed in salt affected areas of Pakistan. In saline environment, plants take up excessive amount of Na^+ at the cost of K^+ and Ca^{++} (Kuiper, 1984). The accumulation of Cl^- parallels that of Na^+ (Yeo and Flowers, 1985) and three ions which commonly cause osmotic problems and specific ion toxicity were Cl^- and SO_4^{2-} with Na^+ associations, though Ca^{++} and Mg^{++} may also be involved in this process (Fitter and Hay, 1981). Generally Cl^- salts are considered more toxic than SO_4^{2-} salts for plant growth. The reason for toxicity under saline conditions has been assigned to certain ions excesses (Yeo and Flowers, 1984). All sensitive varieties tend to accumulate high amounts of Na^+ and Cl^- in developing younger leaves while that of tolerant varieties accumulate these ions in older leaves. The tolerant plants are able to retranslocate K^+ more efficiently from older to developing leaves, exchanging them with sodium ions and thus maintaining favourable K^+/Na^+ ratios in

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young leaves and stems (Bogemans and Stassart, 1987). On saline soils, a normally grown sunflower crop takes up large quantities of Na^+ as well as considerable amount of Cl^- (Bhatt and Indirakutty, 1973). The K^+ content was significantly increased in sunflower at moderate salinity (Shimose, 1973; Heikal, 1977) but reduced drastically at higher salinity level (Heikal, 1977). The objectives of this study were to determine the effect of various salinity levels on ions uptake in the leaves contributing to yield, to develop criteria of selection for sunflower and to mark the salt tolerant genotypes in the available germplasm.

MATERIALS AND METHODS

Three salinity levels were induced artificially in polyethylene lined cylindrical pots, having internal diameter and depth of 26 cm, to determine the influence of salinity on ions accumulation. The experiment was laid out in the randomized complete block design in three replicates. Each pot was filled with 8 kg of air dried, ground and sieved non-saline sand and soil mixture in the ratio of 3:1. The media were salinized before the sowing of seed to give EC values 5 and 10 dSm^{-1} at 25°C by using NaCl, Na_2SO_4 , CaCl_2 and MgSO_4 in the ratio of 5:9:5:1, respectively. The $\text{EC}_e : 2\text{dSm}^{-1}$ of sand and soil mixture was kept as control. Canal water ($\text{EC}:0.29 \text{dSm}^{-1}$) was applied for irrigation throughout the experiment and whenever required. Nitrogen and phosphorus fertilizer was calculated at 86:62 kg per hectare, respectively (Chaudhry, 1986). Complete phosphorus and a half of nitrogen were added at sowing and the rest of nitrogen was applied at flowering. In addition to the recommended dose of nitrogen, urea was applied continuously throughout the experiment twice a week in the form of solution using 5g of urea in 5 litre of canal water and applying 300 ml per pot because of the exhaustive nature of sunflower. Topmost fully expanded leaf whorls were collected at the initiation of flowering for Na^+ , K^+ and Cl^- accumulation analysis in tissue sap using standard techniques and one plant of each sunflower genotype served as a replicate. Chloride ions in the tissue sap was determined using Corning Chloride Analyser 925, Corning Limited Halstead Essex, England, directly calibrated in mole m^{-3} . Na^+ and K^+ were determined in ppm using Petracourt PFPI Flame Photometer. The data were subjected to analysis of variance technique (Steel and Torrie, 1980) and individual comparisons were made using Duncan's new multiple range test (Duncan, 1955) at 0.05 level of probability.

RESULT AND DISCUSSION

Ions accumulation in sunflower leaves at flower initiation exhibited significant ($P \leq 0.01$) differences between the salinity levels for all parameters. Differences among genotypes were found significant at 0.01 probability level for Na^+ accumulation, K^+/Na^+ ratio and significant at 0.05 probability level for K^+ accumulation but non-significant for Cl^- accumulation in leaves of sunflower genotypes was found non-significant for all parameters studied.

Mean Cl^- accumulation (Table 1) in the sunflower genotypes increased non-significantly by 9.7 percent at $\text{EC}:5 \text{dSm}^{-1}$ but significantly by 33.5 percent at $\text{EC}:10 \text{dSm}^{-1}$

as compared with the control. All sunflower genotypes were non-significantly different from each other at all salinity levels.

Table 1. Mean chloride ions accumulation in sunflower leaves at different salinity levels with percent increase(+)/decrease(-) over control.

| Genotype | Salinity level | | | Mean |
|---------------|----------------|-----------------|------------------|--------|
| | T ₀ | T ₁ | T ₂ | |
| 1. S-82 | 56.667 | 53.000(-6.47) | 73.000(+30.43) | 60.889 |
| 2. SF-103 | 80.667 | 65.667(-19.59) | 93.333(+15.70) | 79.889 |
| 3. Suncom-90 | 61.667 | 54.00(-12.43) | 85.333(+38.38) | 67.000 |
| 4. Suncom-110 | 59.333 | 82.667(+39.33) | 81.00(+36.52) | 74.333 |
| 5. MC-212 | 32.333 | 48.667(+50.52) | 104.000(+221.65) | 61.667 |
| 6. Romania | 67.667 | 62.667(-7.39) | 96.667(+42.86) | 75.667 |
| 7. Dekalb | 74.33 | 70.333(-5.38) | 97.333(+30.94) | 80.667 |
| 8. KN-1 | 77.33 | 90.333(+16.81) | 86.667(+12.10) | 84.778 |
| 9. KN-2 | 75.000 | 60.667(-19.11) | 69.000(-8.0) | 68.222 |
| 10. RO-53 | 53.000 | 116.00(+118.86) | 78.333(+47.80) | 82.444 |
| 11. RO-150 | 61.000 | 52.00(-14.75) | 80.667(+32.24) | 64.556 |
| 12. DM-2 | 67.000 | 61.333(-8.45) | 75.000(+11.94) | 67.778 |
| 13. Record | 44.667 | 75.333(+68.65) | 85.000(+90.30) | 68.333 |
| 14. Vniimk | 73.000 | 89.667(+22.83) | 85.667(+17.35) | 82.778 |
| 15. IS-3107 | 84.000 | 79.000(-5.95) | 101.000(+20.23) | 88.000 |
| Mean | 64.511 b | 70.756 b(+9.68) | 86.133 a(+33.52) | |

Figures sharing similar letters are non-significantly different at 0.05 probability level.

Mean values of Na⁺ accumulation (Table 2) in the uppermost leaf whorls of sunflower genotypes decreased by 29.6 and 20.3 percent at EC:5 and 10 dSm⁻¹, respectively, as compared with the control, but Na⁺ accumulation increased non-significantly by 13.2 percent at EC:10 dSm⁻¹ as compared with EC:5 dSm⁻¹. The genotype MC-212 (-61.4%) at EC:5 dSm⁻¹ and Dekalb (-77.4%) SF-103(-38.3%), RO-150(-37.5%) and MC-212(-37.4%) at EC:10 dSm⁻¹ showed maximum decrease in Na⁺ contents in leaves as compared with the control and thus showed tolerance to salinity by the ability to maintain Na⁺ at lower level in young growing leaves. However, the genotypes, Vniimk (+0.2%) and RO-53(+5.4%) at EC:5 dSm⁻¹ and Vniimk (+41.9%) and Romania (+5.5%) at EC:10 dSm⁻¹ showed increase in Na⁺ accumulation in leaves, as compared with the control, indicating their sensitivity to Na⁺.

Mean values for K⁺ (Table 3) accumulation in sunflower genotypes increased significantly by 9.8 percent at EC:5 dSm⁻¹ but then decreased by 6.1 percent at EC:10 dSm⁻¹, as compared with the control. However, at EC:5 dSm⁻¹, two genotypes MC-212 (-6.7%) and Suncom-90 (-16.1%) showed decrease in K⁺ accumulation in leaves while at EC:10 dSm⁻¹, all genotypes showed decrease in K⁺ accumulation except four genotypes S-82(+2.6%) RO-150(+3.0%), DM-2(+8.9%) and MC-212(+12.9%) which showed increase in K⁺ accumulation in leaves as compared with the control. Thus, the genotypes

Table 2. Mean sodium ions accumulation in sunflower leaves at different salinity levels with percent increase(+)/decrease(-) over control.

| Genotype | Salinity level | | | Mean |
|---------------|----------------|-------------------|------------------|-------------|
| | T ₀ | T ₁ | T ₂ | |
| 1. S-82 | 151.8 | 130.533(-14.10) | 134.933(-11.93) | 139.089 bc |
| 2. SF-103 | 134.933 | 102.667(-23.91) | 83.233(-38.31) | 106.944 cd |
| 3. Suncom-90 | 169.033 | 93.700(-44.57) | 115.500(-31.67) | 126.078 bcd |
| 4. Suncom-11- | 148.500 | 85.067(-42.71) | 134.933(-13.67) | 122.833 cd |
| 5. MC-212 | 128.333 | 49.500(-61.43) | 80.300(-37.42) | 86.044 d |
| 6. Romania | 99.367 | 58.667(-40.96) | 104.867(+5.53) | 87.633 d |
| 7. Dekalb | 211.567 | 160.600(-24.09) | 134.200(-77.37) | 168.789 ab |
| 8. KN-1 | 212.667 | 135.667(-36.21) | 164.633(-22.59) | 170.989 ab |
| 9. KN-2 | 157.667 | 118.067(-25.12) | 140.067(-11.16) | 138.600 bc |
| 10. RO-53 | 203.867 | 214.867(+5.39) | 160.600(-21.22) | 193.111 a |
| 11. RO-150 | 165.00 | 87.267(-47.11) | 103.033(-37.55) | 118.433 cd |
| 12. DM-2 | 140.800 | 79.933(-43.23) | 106.333(-24.48) | 109.022 cd |
| 13. Record | 116.233 | 100.467(-13.56) | 117.333(+0.95) | 111.344 cd |
| 14. Vniimk | 83.967 | 85.800(+2.18) | 119.167(+41.92) | 96.311 cd |
| 15. IS-3107 | 123.367 | 79.567(-35.50) | 92.033(-25.39) | 98.322 cd |
| Mean | 149.807 a | 105.491 b(-29.58) | 119.411 b(20.29) | |

Genotypes sharing similar letters are non-significantly different at 0.05 probability level.

Table 3. Mean potassium ions accumulation in sunflower leaves at different salinity levels with percent increase(+)/decrease(-) over control

| Genotype | Salinity level | | | Mean |
|---------------|----------------|-----------------|-----------------|----------|
| | T ₀ | T ₁ | T ₂ | |
| 1. S-82 | 6864.6 | 7109.4(+3.57) | 7044.8(+2.62) | 7006.3 b |
| 2. SF-103 | 6885.0 | 7558.2(+9.77) | 5909.2(-14.17) | 6484.1 b |
| 3. Suncom-90 | 7986.6 | 6701.4(-16.09) | 7500.4(-6.09) | 7396.1 b |
| 4. Suncom-110 | 6919.0 | 7119.6(+2.90) | 6555.2(-5.26) | 6864.6 b |
| 5. MC-212 | 6732.0 | 6262.8(-6.97) | 7605.8(+12.98) | 6866.9 b |
| 6. Romania | 6609.6 | 7877.6(+19.84) | 5946.6(-10.03) | 6811.3 b |
| 7. Dekalb | 7537.8 | 7741.8(+2.71) | 6242.4(-17.18) | 7174.0 b |
| 8. KN-1 | 8534.0 | 9953.4(+16.63) | 8156.6(-4.30) | 8881.3 a |
| 9. KN-2 | 6898.6 | 8330.0(+20.75) | 6803.4(-1.78) | 7344.0 b |
| 10. RO-53 | 6721.7 | 9978.3(+48.45) | 6252.6(-6.98) | 7650.9 b |
| 11. RO-150 | 7286.2 | 7435.8(+2.05) | 7507.2(+3.03) | 7409.7 b |
| 12. DM-2 | 6820.4 | 8421.8(+23.48) | 7432.3(+8.97) | 7558.2 b |
| 13. Record | 7027.8 | 7422.2(+5.61) | 5984.0(-14.85) | 6811.3 b |
| 14. Vniimk | 7347.4 | 8182.6(+10.55) | 5637.2(-23.28) | 7035.7 b |
| 15. IS-3107 | 7629.6 | 8319.8(+9.05) | 6626.6(-13.15) | 7525.3 b |
| Mean | 7186.7 b | 7890.4 a(+9.79) | 6746.9 b(-6.12) | |

Figures sharing similar letters are non significantly different at 0.05 probability level.

S-82, RO-150, DM-2 and MC-212 showed the ability to maintain K^+ at a high level in developing leaves by a mechanism of retranslocation.

Mean K^+/Na^+ ratio (Table 4) in sunflower genotypes increased significantly by 71.6 percent at $EC:5 \text{ dSm}^{-1}$ and non-significantly by 22.5 percent at $EC:10 \text{ dSm}^{-1}$ as compared with the control. Mean value for K^+/Na^+ ratio in sunflower genotypes decreased by 28.6 percent at $EC:10 \text{ dSm}^{-1}$ as compared with $EC:5 \text{ dSm}^{-1}$. At $EC:5 \text{ dSm}^{-1}$, all genotypes showed increase in K^+/Na^+ ratio as compared with the control and the genotypes MC-212, DM-2 and KN-1 were leading with the increase of 149.9, 118.2 and 104.4 percent, respectively. Similarly, at $EC:10 \text{ dSm}^{-1}$, all genotypes showed increase in the K^+/Na^+ ratio with MC-212 showing maximum increase of 98.4 percent followed by KN-2(+54.0%), S-82(+45.2%), RO-150(+44.75%) and KN-1(+44.63%). However, two genotypes, Vniimk (-44.9%) and Record (-15.9%), showed decrease in K^+/Na^+ ratio in leaves as compared to with the control. The sunflower genotypes showing tolerance to salinity maintained the K^+/Na^+ ratio at a high level in younger leaves either by decreasing Na^+ , increasing K^+ , or both.

Table 4. Mean K^+/Na^+ ratio in sunflower leaves at different salinity levels with percent increase(+)/decrease(-) over control.

| Genotype | Salinity level | | | Mean |
|---------------|----------------|------------------|------------------|-------------|
| | T ₀ | T ₁ | T ₂ | |
| 1. S-82 | 47.453 | 54.473(+14.79) | 68.913(+45.22) | 56.947 cd |
| 2. SF-103 | 52.567 | 79.587(+51.40) | 74.170(41.10) | 68.774 abcd |
| 3. Suncom-90 | 52.057 | 84.250(+61.84) | 65.387(+25.61) | 67.231 abcd |
| 4. Suncom-110 | 46.533 | 86.197(+85.24) | 49.937(+7.31) | 60.889 bcd |
| 5. MC-212 | 53.720 | 134.247(+149.90) | 106.587(+98.41) | 98.184 a |
| 6. Romania | 67.663 | 134.490(+98.76) | 69.197(+2.27) | 90.450 ab |
| 7. Dekalb | 37.953 | 75.723(+99.52) | 50.747(+33.71) | 54.808 cd |
| 8. KN-1 | 40.310 | 82.410(+104.40) | 58.300(+44.63) | 60.340 bcd |
| 9. KN-2 | 45.927 | 78.583(+71.10) | 70.73(+54.00) | 65.080 bcd |
| 10. RO-53 | 33.140 | 45.423(+37.06) | 42.193(+26.50) | 40.252 d |
| 11. RO-150 | 50.647 | 87.833(+73.42) | 73.313(+44.75) | 70.598 abcd |
| 12. DM-2 | 53.120 | 115.887(+118.16) | 70.017(+31.81) | 79.674 abc |
| 13. Record | 61.097 | 79.910(+30.79) | 51.383(-15.90) | 64.130 bcd |
| 14. Vniimk | 88.937 | 105.467(+18.59) | 48.977(-44.93) | 81.127 ab |
| 15. IS-3107 | 63.223 | 106.907(+69.09) | 73.113(+15.64) | 81.081 abc |
| Mean | 52.956 b | 90.864 a(+71.58) | 64.864 b(+22.49) | |

Figures sharing similar letters are non-significantly different at 0.05 probability level.

In general, Cl^- accumulation in sunflower leaves increased with increase in salinity but there were non-significant differences between the sunflower genotypes. Na^+ accumulation significantly decreased at moderate salinity but then increased at high salinity level as compared with moderate salinity. Sunflower genotypes showed ability to regulate Na^+ at lower level in young growing leaves. K^+ accumulation in leaves increased at moderate salinity but then decreased at high salinity level and exactly similar behaviour was observed in the case of K^+/Na^+ ratio in sunflower leaves. The genotypes MC-212,

RO-150, DM-2 and IS-3107 at EC:5 dSm⁻¹ and MC-212, Dekalb, SF-103, and RO-150 at EC:10 dSm⁻¹ maintained Na⁺ at a low level while the genotypes RO-53, KN-1, DM-2 and IS-3107 at EC:5 dSm⁻¹ and MC-212, KN-1, RO-150, DM-2, S-82 and Suncom-90 at EC:10 dSm⁻¹ accumulated more K⁺ in young leaves. The genotypes Romania, MC-212, DM-2, KN-1, IS-3107 and Vniimk at EC:5 dSm⁻¹ and MC-212, IS-3107, RO-150, SF-103, DM-2 and KN-2 at EC:10 dSm⁻¹ showed a better K⁺/Na⁺ ratio in the uppermost leaf whorl in sunflower. In sunflower, the genotypes maintaining Na⁺ at a low level and accumulating more K⁺, thus giving favourable K⁺/Na⁺ ratio in young growing leaves, are tolerant to salinity. Therefore, on the basis of a chemical analysis of tissue sap, the genotypes DM-2 and KN-1 were moderately tolerant while MC-212, RO-150 and IS-3107 were tolerant to high salinity level.

The genotypes which showed tolerance to salinity or a mechanism of adaptation to excessive soluble salts in their rhizosphere in present studies are getting support from the earlier research findings which reported higher yield production or minimum yield losses at high level of salinity by sunflower genotypes RO-150 and IS-3107 (Hussain and Rehman, 1992) and better performance in growth and development stages by genotype MC-212 (Rehman and Hussain, 1992), which may be further utilized in breeding programmes. Therefore, it is concluded that sunflower genotypes RO-150, IS-3107 and MC-212 were tolerant to high levels of salinity.

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MEJORA DEL GIRASOL PARA TOLERANCIA A LA SALINIDAD: BASE FISIOLÓGICA DE LA TOLERANCIA EN GIRASOL (*Helianthus annuus* L.)

RESUMEN

Quince genotipos de girasol fueron estudiados para acumulación de iones en sus hojas. Un alto grado de variabilidad genética fue encontrado entre genotipos de girasol para acumulación de iones. La acumulación de iones de cloro se incrementó con salinidad moderada y alta. La acumulación de iones de sodio se incrementó a niveles más altos de salinidad mientras que la acumulación de iones de potasio se incrementó primeramente con salinidad moderada y entonces disminuyó con niveles más altos de salinidad. Un comportamiento similar fue observado para el cociente K^+/Na^+ en hojas jóvenes a niveles altos y moderados de salinidad. Los genotipos de girasol mostraron tolerancia a la salinidad mediante el mantenimiento del Na^+ a bajos niveles y el cociente K^+/Na^+ a niveles más altos en hojas jóvenes en crecimiento. Esto fue considerado como un buen criterio de selección para tolerancia a la salinidad en girasol.

SELECTION DU TOURNESOL À LA TOLÉRANCE À LA SALINITÉ: PHÉNOMÈNES PHYSIOLOGIQUES IMPLIQUÉS DANS LA TOLÉRANCE À LA SALINITÉ CHEZ LE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

L'accumulation d'ions dans les feuilles de tournesol a été étudiée chez quinze génotypes. Un haut degré de variabilité génétique a été mise en évidence parmi ces génotypes. L'accumulation d'ions chlorides s'amplifie en présence de salinités modérées mais croît pour des niveaux de salinités supérieurs alors que l'accumulation des ions potassium augmente d'abord sous l'action de salinités moyennes puis décroît lorsque celles-ci deviennent plus importantes. Un comportement identique a été noté concernant le rapport K^+/Na^+ pour des niveaux de salinité moyens et élevés. Les génotypes de tournesol étudiés manifestent une tolérance à la salinité en maintenant les concentrations en sodium à des niveaux plus faibles et le rapport K^+/Na^+ à des seuils plus élevés dans les jeunes feuilles en croissance. Nous avons trouvé que cela pourrait constituer un bon critère pour la tolérance à la salinité chez le tournesol.