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INHERITANCE OF SALT TOLERANCE IN SUNFLOWER

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SUMMARY

High soil salinity occurs in a large area where sunflower (*Helianthus annuus* L.) is produced in the USA and limits production capabilities there and in many parts of the world where sunflower is grown, particularly under irrigated conditions in arid climates. A wild species of sunflower, *Helianthus paradoxus* Heiser, was noted to be unusually tolerant to saline conditions. This investigation examined the salinity tolerance of five interspecific germplasm lines derived from crosses of *H. paradoxus* with cultivated *H. annuus* and studied the inheritance of salinity tolerance. Preliminary results indicated that three interspecific crosses had tolerance to a 15 (EC_e^3 24.7 dSm^{-1}) g/l NaCl solution in both a seedling and germination test. These germplasm lines and a susceptible inbred line were crossed. Segregation ratios of F_2 and backcross generations indicated that one major dominant gene, proposed as Sa_1, controlled seedling tolerance to salinity. A recessive modifier gene also appeared to be affecting seedling tolerance to salinity. In the germination study, no single gene was clearly identified for control of tolerance to salinity, however, a level of tolerance was found and appeared to be dominantly controlled. These seedling and germination procedures will be further tested to determine their utility in breeding programs desiring increased tolerance to soil salinity.

Key words: Sunflower, salt tolerance, genetics, breeding, wild *Helianthus* species.

INTRODUCTION

High soil salinity generally occurs in areas where groundwater discharges by evapotranspiration from a shallow water table, resulting in salts precipitating and accumulating on or near the soil surface (Seeling and Richardson, 1991). High salinity can be found in both fine textured or coarse textured soils, depending on the depth of the water table and rate of evapotranspiration. Compaction may also intensify soil salinity by increasing upward capillary movement of water, thereby increasing groundwater discharge. Soil survey data of North Dakota indicated that approximately 280,000 hectares are affected by salinity in 34 of 52 counties, representing about 9% of the hectares of this 34 county area. Much of the affected area occurs where sunflower (*Helianthus annuus* L.) is a major crop.

Soil salinity also occurs in many parts of the world, and is a particularly serious problem in arid or semi-arid areas where irrigation is required (Bhatt and Indirakutty, 1973; Maas and Hoffman, 1976; Hussain and Rehman, 1993). Most water from sources other than precipitation contains dissolved salt, and rate of salinization depends directly on the amount and quality of water utilized. Salt accumulation is one of the major factors

limiting utilization of new lands for agricultural purposes in many countries. Identification of crop species tolerant to salt and/or development of salt tolerant cultivars of established crop species is a major objective of research in these countries.

Sunflower is generally considered moderately tolerant to soil salinity (Hardwick and Ferguson, 1978; Blamey et al., 1986). However, one species, *Helianthus paradoxus* Heiser, was noted to be unusually tolerant to saline conditions (Seiler et al., 1981). *H. paradoxus* is a rare and endangered species, with limited adaptation to Texas and New Mexico. Preliminary studies with sunflower indicated that *H. paradoxus* and one species of *H. annuus* L. (*H. jaegeri* Heiser) had tolerance to salt concentrations ranging from EC_e^3 14.6 dSm^{-1} to EC_e^3 23.4 dSm^{-1} in hydroponic culture (Chandler and Jan, 1984). Some plants of *H. paradoxus* survived at EC_e^3 46.8 dSm^{-1}. The salt tolerance in *H. paradoxus* was found to be dominant with hybrids between *H. paradoxus* and cultivated sunflower surviving as well as the wild parent. Five interspecific crosses utilizing *H. paradoxus* were released in 1989 (Seiler, 1991). These lines may have genetic characteristics which contribute to salinity tolerance. The objectives of this investigation were to test the salinity tolerance of the five interspecific crosses and to study the inheritance of salinity tolerance in sunflower.

MATERIALS AND METHODS

The five germplasms, plant introduction (PI) number, and collection site of *H. paradoxus* accessions utilized in this investigation were: PAR-1084-1 (PI 46880, Leon Lake Dam, Ft. Stockton, TX), PAR-1671-1 and PAR-1671-2 (PI 468801, Dexter, NM), and PAR-1673-1 and PAR-1673-2 (PI 468802, Ft. Stockton, TX) (Seiler, 1991).

Plant sensitivity to salinity was reported to vary from one growth stage to the next among crop species (Maas and Hoffman, 1976). In their studies, cereal crops tended to be more sensitive than dicotyledon crops during emergence and early seedling growth than during germination and later stages of growth. In contrast, sugarbeet and other dicotyledon crops were most sensitive during germination. Therefore, two studies were designed for sunflower; a seedling test and a germination test.

Seedling Test

Seeds of HA 821, a cultivated sunflower line susceptible to salt, and the five *H. paradoxus* (PAR) germplasm sources were germinated on wet paper towels. After three days, seedlings with a 20 mm primary root length were planted into 10 cm tall paper cups containing 150 ml of sandy loam soil (EC_e^3 1.15 dSm^{-1}). The seedlings were watered with distilled water each day until the first pair of true leaves reached 2.5 cm in length. At this stage, a salt (NaCl) solution of 0 (EC_e^3 0.002 dSm^{-1}), 23.4 (EC_e^3 36.9 dSm^{-1}), and 46.8 (EC_e^3 65.7 dSm^{-1}) g NaCl/liter of distilled water was used to water the seedlings. Approximately 15 ml of the solution was used each day. After 14 days, seedlings were cut at the soil surface and fresh weight recorded.

Based on the preliminary studies, these NaCl solutions were determined to be too concentrated as plant growth after 8 days was severely stunted in both the *H. paradoxus* germplsam lines and HA 821. A second study was initiated utilizing a 0 (EC_e^3 0.002 dSm^{-1}) and 15 (EC_e^3 24.7 dSm^{-1}) g/l NaCl solution to test the same lines. The 15 g/l NaCl

solution clearly differentiated the germplasm lines, PAR-1671-1, PAR-1673-1, and PAR-1673-2, and the susceptible check, HA 821, for tolerance to salt.

PAR-1671-1, PAR-1673-1 and PAR-1673-2 were crossed with HA 821. F_1 plants were backcrossed to HA 821 and their respective PAR line, and self-pollinated. The following generations were utilized in the inheritance investigation: parents, F_1, F_2, BC_1F_1(P1), and BC_1F_1(P2). HA 821 was designated P1 and the PAR parental line was designated P2. The number of seedlings observed was 40 for parents and F_1 generations, 140-240 for the F_2 generations, and 40-60 for the backcross generations, depending on the number of available seeds.

Procedures for the inheritance study were the same as the parental test, utilizing 0 and 15 g/l NaCl solutions and a 14 day treatment. After 14 days, seedlings were cut at the soil surface and fresh weight measured. The fresh weights were plotted and classes determined. The rations of tolerant (T) to susceptible (S) were used to determine the inheritance patterns. The data for each F_2 and backcross family were analyzed utilizing Chi-square tests for goodness of fit and heterogeneity.

Germination Test

Paper towels were wetted with 0 (EC_e^3 0.002 dSm^{-1}), 23.4 (EC_e^3 36.9 dSm^{-1}), and 46.8 (EC_e^3 65.7 dSm^{-1}) g salt (NaCl)/liter of distilled water. Seeds of HA 821 (control) and the five *H. paradoxus* germplasm lines were placed on the towels. Seeds were hand picked so that only seeds of nearly equal size were used in all treatments. The towels were rolled and sealed in a plastic bag to prevent evaporation and held for 6 days at 21°C in the dark. After 6 days, germination was observed. Very little growth of the primary root was observed in all materials. A second study utilizing the same lines was used with a 0 (EC_e^3 0.002 dSm^{-1}), 10 (EC_e^3 17.5 dSm^{-1}), 15 (EC_e^3 24.7 dSm^{-1}), and 20 (EC_e^3 32.3 dSm^{-1}) g/l NaCl solution for 6 days. Differentiation between HA 821 and the lines PAR-1671-1, PAR-1673-1, and PAR-1673-2 occurred with the 15 and 20 g/l solution. The 15 g/l solution was chosen for the inheritance study.

Parents, F_1, F_2, and backcross generations previously described were tested for germination using the above method. The lengts of the primary root was measured and recorded 6 days after germination. The lengths were plotted and classes determined. The data for each F_2 and backcross family were analyzed utilizing Chi-square tests for goodness of fit and heterogeneity.

RESULTS AND DISCUSSION

Seedling study

The average fresh weights of HA 821, PAR-1671-1, PAR-1673-1, and PAR-1673-2, seedlings treated with distilled water (0 NaCl) were 2.48, 2.42, 2.56, and 2.36 g, respectively (Table 1). The average fresh weights of the same lines treated with the 15 g/l NaCl solution indicated that the NaCl solution reduced growth of the check, HA 821, substantially. HA 821 seedlings averaged 0.90 g fresh weight with a range of 0.37 to 1.27 g. Plants weighing less than 0.61 g were nearly dead with leaves wilted and dried. The plants of PAR-1671-1, PAR-1673-1, and PAR-1673-2, although slightly reduced in fresh weight, remained green and vigorous, and had only slightly wilted leaves. Seedling wight of F_1

Table 1. Average fresh weight (g) of seedlings tested with a 0 and 15 g/l NaCl concentracion after 14 days of treatment.

	P1		P2					
	HA 821		PAR 1671-1		PAR 1673-1		PAR 1673-2	
Generation	0	15	0	15	0	15	0	15
Parent	2.48	0.90	2.42	1.89	2.56	1.75	2.36	1.84
F_1			2.91	2.28	3.07	1.96	2.83	2.09
F_2			2.61	1.99	2.68	1.83	2.84	1.90
BC_1F_1 (P1)			2.54	1.21	2.58	1.28	2.66	1.18
BC_1F_1 (P2)			2.51	1.68	2.62	1.52	2.60	1.66

P1 = Susceptible parent, P2 = Tolerant parent.

Table 2. Chi-square analysis for the excepted rations of 3:1 tolerant: susceptible for F_2 populations and 1:1 tolerant: susceptible for backcross populations tested with a NaCl concentration of 15 g/l in a seedling growth study.

	F_2					Backcross				
	No. of seedlings					No. of seedlings				
Cross	T	S	Total	X^2	P	T	S	Total	X^2	P
1	93	24	117	1.26	0.30-0.20	16	24	40	1.60	0.30-0.20
2	84	33	117	0.64	0.50-0.30	15	22	37	1.32	0.30-0.20
3	93	27	120	0.40	0.70-0.50	18	22	40	0.40	0.70-0.50
X^2d	270	84	354	0.31	0.70-0.50	49	68	117	3.08	0.10-0.05
X^2b				1.99	0.50-0.30				0.24	0.90-0.70

T = Tolerant, S = Susceptible
P = Probability.

Table 3. Average length of primary root (mm) of seedlings tested with a and 15 g/l NaCl concentration after 6 days of treatment.

	P1		P2					
	HA 821		PAR 1671-1		PAR 1673-1		PAR 1673-2	
Generation	0	15	0	15	0	15	0	15
Parent	43.3	2.6	48.1	21.2	51.3	14.5	45.5	16.8
F_1			60.1	13.7	76.2	16.8	70.2	13.5
F_2			57.5	23.8	70.1	28.9	66.5	27.8
BC_1F_1 (P1)			44.4	22.4	52.0	12.9	51.1	15.7
BC_1F_1 (P2)			46.6	23.1	59.2	23.8	53.9	22.9

P1 = Susceptible parent, P2 = Tolerant parent.

Table 4. Chi-square analysis for the excepted rations of 9:7 and 3:1 tolerant: susceptible for F_2 populations and 1:3 and 1:1 tolerant: susceptible for backcross populations tested with a NaCl concentration of 15 g/l, germination study.

	F_2							Backcross						
	No. of seedlings			X^2	P	X^2	P	No. of seedlings			X^2	P	X^2	P
Cross	T	S	Total	9:7		3:1		T	S	Total	1:3		1:1	
1	145	104	249	0.41	0.7-0.5	37.33	<0.01	-	-	-	-	-	-	-
2	113	121	234	6.27	0.05-0.01	89.03	<0.01	26	8	34	0.04	0.9-0.7	9.52	<0.01
3	113	58	171	6.70	<0.01	7.25	<0.01	140	59	199	2.29	0.2-0.1	32.96	<0.01

T = Tolerant, S = Susceptible
P = Probability.

plants of the three crosses HA 821/PAR-1671-1, HA 821/PAR-1673-1, and HA 821/PAR-1673-2 were equal or slightly higher in fresh weight than their PAR parental lines when treated with the 15 g/l NaCl solution. Variability among plants in the F_2 generations for seedling fresh weight was considerable, ranging from severely stunted plants to plants equal in vigor to F_1 or parental plants.

Plotting fresh weight of plants in the F_2 generations resulted in two classes, determined to be tolerant and susceptible classes when compared with the parental lines. However, the tolerant class had lower fresh weights, in general, than F_1 or parental PAR plants. The number of plants in each class were counted and a 3:1 tolerant:susceptible ratio was tested utilizing a Chi-square analysis (Table 2). The data for all three crosses were non-significant for the 3:1 ratio indicating a one gene control. Data were pooled and the deviation and heterogeneity Chi-square were non-significant, indicating that all three germplasm lines had one major gene controlling salt tolerance in these lines of sunflower.

The backcross progeny of F_1 plants crossed to the susceptible parent, HA 821, segregated into distinct tolerant and susceptible classes. The susceptible plants had lower fresh weights than tolerant plants and had dry wilted leaves. Weights of the tolerant class were generally lower than the F_1 or parental PAR fresh weights. The number of plants in each class were counted and a 1:1 tolerant:susceptible ratio was tested utilizing a Chi-square analysis (Table 2). Crosses were non-significant for the 1:1 ratio; however, the fit for cross 1 was very weak with more susceptible plants than expected. Data were pooled and the deviation and heterogeneity Chi-square indicated a weak fit to a 1:1 ratio.

The backcross generations of the F_1 crossed to the resistant PAR parent did not segregate into distinct classes and there was little variation among plants. Although the plants were vigorous, they had less fresh seedling weights than their respective F_1 and PAR parents.

Based on the analysis of the F_2 and backcross generations, it appears that there is one major dominant gene controlling salt tolerance in the *H. paradoxus* crosses of sunflower. The proposed designation of this gene is Sa_1. However, since tolerant plants in the F_2 generation had seedling weights generally lower than seedling weights of F_1 or parental PAR plants and both backcrosses were skewed toward the susceptible parent, it appears that a recessive modifier gene also may be influencing susceptibility to salt.

Germination Test

Primary root lengths of HA 821, PAR-1671-1, PAR-1673-1, and PAR-1673-2 treated with 0 g/l NaCl solution averaged 43.3, 48.1, 51.3, and 45.5 mm, respectively (Table 3). The average root lengths of the same lines treated with 15 g/l NaCl solution indicated that the NaCl solution reduced primary root length in all populations. HA 821 had very little root growth, averaging only 2.6 mm in length, whereas the root lengths of PAR-1671-1, PAR-1673-1, and PAR-1673-2 were 21.2, 14.5, and 16.8 mm, respectively. F_1 plant root lengths of crosses between HA 821 and the PAR lines had nearly the same or slightly shorter root lenghts than the PAR parental lines. The F_2 generation segregated into resistant and susceptible classes when compared to the parental lines. However, the three crosses differed in their numbers in each class. The data for cross 1 fit a 9:7 tolerant:susceptible ratio indicating control by two complementary dominant genes

(Table 4). The data for crosses 2 and 3 did not fit a 9:7 ratio and segregation in all three crosses did not fit a 3:1 ratio. The backcross of the cross 1 F_1 to the susceptible parent, HA 821, did not segregate into distinct classes and the population was normally distributed. Only the data for cross 2 fit a 1:3 tolerant:susceptible ratio expected for a two complementary dominant gene model.

The segregation ratios observed in the F_2 and BC_1F_1(P2) generations in the germination test indicate that mode of genetic control for tolerance to NaCl could not be clearly determined. The plant root lengths of the F_1 and backcross generations utilizing the resistant PAR genotypes as recurrent parents were equal to or higher than those of the tolerant parents indicating that genetic control of tolerance was dominant. Several seedlings of the F_2 and backcross generations were very tolerant to NaCl and achieved root lengths of 54 to 66 mm. Even though a clear inheritance pattern controlling NaCl tolerance for germination was not determined, the procedure may be an effective tool for selecting seedlings with increased tolerance.

Studies are underway to select plants tolerant to salt from the F_2 generations of the three crosses utilizing both the seedling and germination studies. Selected plants will be self-pollinated and progenies tested to determine if the methods were effective in identifying stable, tolerant genotypes. Materials derived from the germination test is effective, this method will be preferred, as less trauma to the plants will result in more healthy seedlings. Further testing will utilize soils from an area high in both chloride and sulfate salts. Testing hybrids will determine if sunflower can be more tolerant to salinity, and indirectly, determine if these hybrids can also be more tolerant to drought effects on slightly saline soils.

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HERENCIA DE LA TOLERANCIA A SALINIDAD EN GIRASOL

RESUMEN

La alta salinidad del suelo tiene lugar en una amplia area de cultivo donde el girasol (*Helianthus annuus* L.) es producido en USA, limitando las capacidades de producción en este area y en muchas partes del mundo donde el girasol es cultivado particularmente bajo condiciones de riego en climas áridos. Una especie silvestre de girasol (*Helianthus paradoxus*) Heiser, resultó ser inusualmente tolerante a condiciones salinas. Este investigación examinó la tolerancia a salinidad de cinco lineas derivadas de cruces interespecificos de *H. paradoxus* con *H. annuus* y estudió la herencia de la tolerancia a salinidad.

Resultados prelíminares indicaron que tres cruces interespecificos tuvieron toleranciaa 15 (EC_e^3 24.7 dSm^{-1}) g/l de solución de NaCl en estado de plántula y un test de germinación. Estas lineas fueron cruzadas con una linea susceptible. Las segregaciones de la F2, retrocruces indicaron que un gen dominante, denominado como Sa_1, controló la tolerancia de las plántulas a salinidad, un gen modificador recisivo parece afectar también la tolerancia a salinidad de las plántulas. En el estudio de germinación, no fue identificado claramente ningún gen controlando la tolerancia a salinidad. Sin embargo, un cierto nivel de tolerancia fue encontrado y apareció estar controlado de forma dominante.

Estas plántulas y procedimientos de germinación serán testados de nuevo para determinar su utilidad en programnas de mejora para incrementar la tolerancia a salinidad del suelo.

HÉRÉDITÉ DE LA TOLÉRANCE À LA SALINITÉ CHEZ LE TOURNESOL

RÉSUMÉ

La salinité élevé du sol est présente dans une partie importante des zones de production du tournesol (*Helianthus annuus* L.) aux USA et limite le potentiel de production dans ces zones là ainsi que dans de nombreuses régions du monde où le tournesol est cultivé, particulièrement en conditions irriguées sous climats secs. Une espèce sauvage de tournesol *Helianthus paradoxus* Heiser, a montré une tolérance inhabituelle aux conditions salines. Cette recherche a consisté à analyser la tolérance à la salinité de cinq lignées interspécifiques issues de croisements d'*H. paradoxus* avec le tournesol cultivé *H. annuus*, et à étudier l'hérédité de la tolérance à la salinité. Les résultats préliminaires indiquent que trois hybrides interspécifiques présentent une tolérance à une solution de 15g/l de NaCl (EC_e^3 24.7 dSm^{-1}) en tests plantules ou de germination. Ces lignées et une lignée fixée sensible ont été croisées. Les ratios de ségrégation dans les générations F2 et backcross indiquent qu'un gène majeur dominant, appelé Sa1, contrôle la tolérance des plantules à la salinité. Un gène modificateur récessif parait également affecter la tolérance des plantules à la salinité. Dans l'étude de germination, aucun gène simple n'a été clairement identifié, cependant, un certain degré de tolérance sous contrôle dominant a été mis en évidence. Ces tests plantules et de germination seront éprouvés pour déterminer leur intérêt dans les programmes de sélection pour une meilleure tolérance aux sols salins.