

# **Diversity of salt tolerance in a germplasm collection of wheat**  *(Triticum* **spp.) \***

# H. I. Sayed

Plant Production Department, College of Agriculture, King Sand University, Riyadh, Saudi Arabia

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Summary. A collection (5,072 lines) of wheat germplasm was screened at the seedling stage for tolerance to salinity concentrations having electrical conductivities of 0.8 (control), 12.5, 18.75 and 25.0 dS/m. Surviving seedlings were expressed for each line as a percentage of the control value. The 442 lines with greater than 70% surviving seedlings were tested for whole-life cycle survival under each salinity condition. The data of the reactions to salinity at both the seedling stage and maturity were used to classify the collection according to: (1) country of origin (2) species and ploidy level The data were then subjected to a diversity analysis using the Shannon-Weaver information index. Seedling stage tolerance to 12.5 dS/cm salinity was widely distributed in the collection (79% of lines), whereas only 9% were tolerant at 25.0 dS/m salinity. The seedling stage tolerance was indicative of maturity tolerance. At the seedling stage, entries from USA and Egypt showed the largest proportions of tolerant lines. In addition, USA, Mexico and Egypt entries exhibited the widest variability. Diversity among regions was greater than among countries within regions, while the diversity among species was greater than among ploidy levels. Tetraploids exceeded hexaploids and diploids in the proportion of tolerant lines and diversity. Wheat-rye derivatives showed a good potential for salt tolerance at early stages. Screening more germplasm from the arid and semi-arid regions especially from countries with salt affected soils was recommended.

**Key words:** Diversity analysis - Salt tolerance - *Triticum -* Wheat - Wheat-rye derivatives

#### **Introduction**

Breeding for salt tolerance in higher plants is widely recognised as an important aspect of improving crop productivity (Epstein et al. 1979). Genetic manipulation, however, requires vast germplasm sources to provide adequate variation for selection (Shannon et al. 1981). The emphasis on increasing crop diversity led to a recognition of the need for extensive screening of germplasm collections and exploring new geographical areas.

Rick (1972) collected a wild species of tomato, *Lycopersicon cheesmanii,* from Isla Isabella Galapagoes near tidal sea water. The progeny of a cross between the wild species ad the cultivated tomato *Lycopersicon esculentum* survived up to 70% (33.9 mS/cm) of sea water concentration (Rush and Epstein 1976).

Epstein and Norlyn (1977) sampled a wide spectrum of barley germplasm for salt tolerance but found that only 0.31% of the original seeds survived the imposed high salinity of 75-90% sea water. They recommended large germplasm collections as an alternative to comparing fewer cultivars. Sayed et al. (1984) screened 5,072 wheat lines for salt tolerance and only 3% of the collection survived salinity conditions of 25 dS/m.

The diversity index has been used widely in ecological studies to evaluate species within communities (Pielou 1969). Marshall and Brown (1975) demonstrated its usefulness for analysis of genetic variation. In recent years, it.has been used in *durum* wheat (Jain et al. 1975), in rice (Holcomb etal. 1977), in safflower (Jain and Wu 1977) and in barley (Tolbert et al. 1979).

This paper reports a diversity analysis of a wheat collection screened for salt tolerance at germination and early seedling stage of development. A second diversity analysis based on whole-life cycle survival also is discussed.

## **Materials and methods**

The material for this study included a total of 5,072 lines of spring wheat and triticale from local and different world col-

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lections. They were obtained from Dr. W. Porter of the USDA Germplasm Institute, CIMMYT, ICARDA, FAO, ICRISAT, and others.

The collection was screened for salt tolerance at germination and seedling stages of development. Small plastic pots were filled with 400 g air dried sandy soil (sand 89%, silt 3% and clay 8%; F.C. 7.8%), arranged on trays equipped with drainage pipes and placed on 3-layer illuminated racks in a controlled temperature laboratory ( $23 \pm 2^{\circ}$ C). Ten kernels from each line were planted 2 cm deep in each pot. Four replications were used for each salinity level.

Sea water from the Persian gulf (109 dS/m) was diluted and incorporated with the equivalent of half strength Hoagland's nutrients to provide three saline solution mixtures with electrical conductivities (EC) of  $12.5$  dS/m (S<sub>1</sub>),  $18.75$  dS/m  $(S_2)$  or 25.0 dS/m  $(S_3)$ . The control  $(S_0)$  solution consisted of the dilution water (0.8 dS/m) with added nutrients. The pots were irrigated every 3 days with an excessive volume of the saline solution (50 ml/pot) to ensure leaching of the soil.

The number of normal healthy seedlings in each pot was determined 20 days after planting. The percentage of surviving seedlings was determined for each line and averaged over replications. To avoid discrepancies caused by differences in seed viability in the original germplasm collection, entries which showed less than  $60\%$  surviving seedlings at S<sub>o</sub> (control) were retested from a new seed increase, and the percentage of surviving seedlings of each line was expressed as a percent of its control value. Accordingly, each line maintained three values that corresponded to the percentages of surviving seedlings at the three salinities  $(S_1, S_2 \text{ and } S_3)$ . A line with 0-40% surviving seedlings was considered non-tolerant (NT), 41-70% was medium tolerant (MT) and 71% or more was considered tolerant (T) to the designated salinity.

The 454 lines classified as tolerant to  $S_3$  were selected for a whole-life cycle survival test. Survival was defined as the ability of the plant to tolerate the salinity imposed throughout its life cycle, e.g. from seed to seed (Epstein etal. 1979). Among the 454 selected lines, 442 were tested in a controlled temperature and humidity greenhouse (20-25 °C and 70-80% RH) during the 1982/83 fall-winter season. Twenty seeds from each line were placed in a single hill on ground beds filled with sandy soil (60 cm deep). The hills were spaced at 25 cm in each direction with a control line every 10 hills. Three sets of 442 lines each were planted and irrigated with  $S_0$  saline solution for 15 days to ensure similar densities. Then, each of the sets was assigned to one of the salinities  $S_1$ ,  $S_2$  or  $S_3$ . The quantity of saline water applied to each salinity was adjusted during the season to provide for controlled leaching.

Plants were examined during various growth stages to determine nonsurvivors. The numbers of surviving lines, i.e. those carrying fertile spikes, were determined at maturity. A spike was considered fertile when it contained at least one kernel.

The nature of inheritance of salt tolerance in wheat is not known. Therefore, in the absence of information about the genotypic structure of the lines, the phenotypic expression of the lines for salt tolerance, i.e. percentage of surviving seedlings or lines, was used in the study. The collection was classified according to the country of origin for each of the three salinities. The 18 entires which were not identified for origin were excluded from the analysis. Another 48 entries from Cyprus, Canada and different countries of Europe and East Africa also were discarded because of the small numbers of entries representing each of these countries. In all, 5,006 entries from 17 different countries were analysed. These countries were grouped into five regions following the system used by Jain et al. (1975) for durum wheat.

A similar classification of the collection was carried out according to species and ploidy levels. A group of 74 lines of hexaploide triticale were classified separately under wheat  $\times$  rye derivatives.

The Shannon-Weaver information index (H') was used in the present analysis of genetic diversity. The index is calculated as:

$$
H' = -\sum_{i=1}^{n} Pi \log_2 Pi
$$

where Pi is the proportion of entries in the i th class of an n class trait (Marshall and Brown 1975). A hierarchical analysis of variance for testing the significance of various components of variation in H' also was carried out.

#### **Results**

## *Distribution of different classes*

The maximum number of entries analysed from each country or species and the distribution of classes within each salinity level are presented in Tables 1 and 2. Collections from Algeria, Moroco and Tunisia were included because of the importance of the North African region, although the numbers of entries were less than desired. Entries from Lebanon, Syria and Mexico were derived mainly from breeding programs.

The nature of the overall distribution of entries for tolerance at the seedling stage differed among the three salinities. Similar skewed frequency distributions occurred for  $S_1$  and  $S_3$  while  $S_2$  showed a relatively normal distribution. The different geographical regions exhibited distributions similar in pattern to the over all distribution. Tolerance was widely distributed throughout the collection (79% of entries) for  $S_1$ , while restricted to one third of the population for  $S_2$ , and to only 9% for the highest salinity level  $(S_3)$ . Among countries with more than 100 entries, USA had the greatest proportions of tolerant entries for  $S_2$  (50%) and  $S_3$  (25%), followed by Egypt with 42% and 20%, respectively.

With the exception of the *dicoccoides* species, all tetraploid wheat and the wheat-rye derivatives exhibited relatively higher proportions of tolerant lines for  $S_3$ than the hexaploid wheat (Table2). Diploids were completely void of tolerant lines at this salinity.

Entries from Turkey, Mexico, USA and Egypt constituted 85% of the 442 lines tested for the whole-life cycle survival (Table3). Tolerance was distributed widely among lines for  $S_1$  (100% survival) and  $S_2$  (94%) survival) salinity levels, whereas for  $S_3$  only one of two entries tested was tolerant (53% survival). The USA had the largest proportion of tolerant lines (84%), and was followed by Mexico (77%), Turkey (72.5%) and Egypt (60%). In addition, hexaploid wheat *(T. aestivum)*  showed a higher proportion (79%) of tolerant lines as compared to tetraploid *durum* wheat (65.5%). This was in contrast to the trend of the seedling stage.

Region/country	$\boldsymbol{N}$	$S_1$			$\mathbf{S_2}$			$S_3$			
		NT	MT	T	NT	MT	T	NT	MT	T	
Middle East:											
Lebanon	130	10	17	73	44	25	31	73	15	12	
Jordan	26	4	11	85	42	27	31	77	11	12	
Palestine	29	7	14	79	41	31	28	90	3	7	
Syria	188	13	12	75	55	25	20	87	$\cdot$ 9	$\overline{4}$	
Turkey	1,905	$\overline{\mathbf{c}}$	16	82	46	24	30	80	11	9	
Region 1	2,278	$\overline{\mathbf{3}}$	16	81	46	25	29	80	11	9	
North Africa:											
Algeria	9	-	11	89	56	22	22	100	<u></u>		
Moroco	6			100	33	-	67	83	17		
Tunisia	9		11	89	56	33	11	100	-		
Egypt	183	3	17	80	33	25	42	71	9	20	
Region 2	207	3	16	81	35	25	40	74	8	18	
Central Asia:											
Saudi Arabia	129	1	5	94	18	43	39	95	4	1	
Iraq	53	15	25	60	58	23	19	89	9	$\overline{c}$	
Iran	169	4	18	78	57	26	17	90	5	5	
Pakistan	9	-	22	78	33	22	45	78	22		
India	636	3	21	76	51	29	20	92	6	$\overline{\mathbf{c}}$	
Region 3	996	3	19	78	48	30	22	92	6	$\overline{2}$	
South Asia:											
Australia	315	6	24	70	48	29	23	89	7	4	
Region 4	315	6	24	70	48	29	23	89	7	$\overline{\mathbf{4}}$	
North America:											
<b>USA</b>	265	2	14	84	18	32	50	52	23	25	
Mexico	945	7	15	78	39	27	34	75	14	11	
Region 5	1,210	6	15	79	34	28	37	70	16	14	
World total	5,006	4	17	79	43	27	30	80	11	9	

Table 1. Proportions of nontolerant (NT), medium tolerant (MT) and tolerant (T) lines for each country and region based on per cent of surviving seedlings to salinities of 12.5  $(S_1)$ , 18.75  $(S_2)$  and 25.0  $d\tilde{S}/m$  (S<sub>3</sub>). N= number of lines tested

Table 2. Proportions of nontolerant (NT), medium tolerant (MT) and tolerant (T) lines for each species and ploidy level based on per cent of surviving seedlings to salinities of 12.5  $(S_1)$ , 18.75  $(S_2)$ and 25.0 dS/m  $(S_3)$ . N= number of lines tested



Table 3. Proportions of nontolerant (NT) and tolerant (T) lines for each country, region, species and ploidy level based on per cent of surviving lines at maturity to salinities of 18.75  $(S_2)$  and 25.0 dS/m  $(S_3)$ . N=number of lines tested (% of surviving lines to  $12.5 \text{ dS/m} = 100\%)$ 

Region/country	Hʻ	$\tilde{H}' \pm SE$			
	$\mathrm{S}_\mathrm{1}$	S,	S,		
Middle East:					
Lebanon	1.10	1.55	1.11	$1.25 \pm 0.15$	
Jordan	0.74	1.56	1.01	$1.10 \pm 0.24$	
Palestine	0.93	1.57	0.56	$1.02 \pm 0.30$	
Syria	0.93	1.44	0.67	$1.01 \pm 0.23$	
Turkey	0.77	1.53	0.92	$1.07 \pm 0.23$	
Region	0.82	1.53	0.92	$1.09 \pm 0.22$	

Table 4. Estimates of the diversity index (H') for various countries and regions; mean diversity  $(\bar{H}')$  and its standard error





#### *Diversity index analyses*

The H' values pooled over salinities, regions, species and ploidy levels showed wide variations (Tables 4-6). The three countries with the highest H' values were USA  $(1.22)$ , Mexico  $(1.20)$  and Egypt  $(1.17)$ . These values did not differ significantly from each other (compared by t test: USA vs Mexico  $t=0.13$ ; USA vs Egypt  $t=0.31$ ; Mexico vs Egypt  $t=0.42$ ;  $t_{0.05,2}=4.30$ ). Saudi Arabia, Iran, and India all had smaller H' values despite reasonable numbers of entries (over 100 each). The North American region seemed to have the greatest variation (1.23) followed by North Africa (1.15), the Middle East (1.09) and South Asia (1.07). Central Asia exhibited the least variability (0.87). USA and Egypt had greater variation for tolerance at  $S_3$  than other countries within their regions.

The wheat-rye derivatives showed the highest H' value (1.22) followed by durum wheat (1.16). Tetraploid species exhibited greater variation than the diploids (1.13) and hexaploids (1.09).

The diversity index (H') values obtained from the whole-life cycle test were generally small with an average of 0.67 compared to 1.11 for seedling survival (Table4). Among countries with large number of entries, Egypt had the widest variation (0.75) followed by Turkey (0.65), Mexico (0.61) and USA (0.51). Tetraploid *durum* wheat maintained superiority in variation over the hexaploid *aestivum* species.

The results of the hierarchical analysis of variance of H' (Table 7) indicated that diversity among regions, as expected, was greater than diversity among countries within regions, (larger mean squares associated with regions). In contrast, the diversity among species within ploidy levels was much greater than the diversity among ploidy levels at the seedling stage. Similar information was not attainable from the whole life cycle test because of the absence of diploids, and most of the tetraploid and hexaploid species.

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Table 5. Estimates of the diversity index (H') for various species and ploidy levels, mean diversity  $(\tilde{H}')$  and its standard error (SE) over salinities of 12.5  $(S_1)$ , 18.75  $(S_2)$  and 25.0 dS/m  $(S<sub>3</sub>)$ . Based on seedling stage data

Ploidy/species	H		$H' \pm SE$			
	$S_1$	$S_{2}$	$S_3$			
Diploids:						
boeticum and топососсит	1.10	1.35	0.87	$1.11 \pm 0.17$		
Total	1.10	1.35	0.87	$1.11 \pm 0.17$		
Tetraploids:						
dicoccoids	0.97	0.72	0.00	$0.56 \pm 0.29$		
dicoccum	0.82	1.47	1.10	$1.13 \pm 0.20$		
durum	0.93	1.57	0.98	$1.16 \pm 0.21$		
turgidum	0.47	1.54	0.47	$0.82 \pm 0.34$		
Others	1.27	1.19	1.14	$1.20 \pm 0.04$		
Total	0.93	1.57	0.98	$1.16 \pm 0.21$		
Hexaploids:						
aestivum	0.82	1.54	0.86	$1.10 \pm 0.22$		
sphaerococcum	0.78	1.53	0.40	$0.90 \pm 0.33$		
compactum	0.00	0.91	0.91	$0.61 \pm 0.30$		
Total	0.82	1.59	0.86	$1.09 \pm 0.25$		
Wheat $\times$ rye						
triticale	0.98	1.56	1.13	$1.22 \pm 0.17$		
Total	0.98	1.56	1.13	$1.22 \pm 0.17$		
Collection	0.87	1.55	0.92	$1.11 \pm 0.22$		

# **Discussion**

The first step in genetic improvement of salt tolerance is finding sufficient variation within the breeding population (Shannon 1978). Hybridisation and selection among agronomic cultivars, and the use of wild relatives to increase variability or to develop new cultivated species is the second step. Epstein etal. (1979) suggested that large amounts of genetically governed variation, with respect to salt tolerance, may exist within a crop. Allard (1970) emphasized that most species probably contain millions of genotypes, yet it is impractical to collect and preserve all existing variants. However, at least one copy of each of the different alleles of the target species should be preserved (Bennett 1970). Therefore, an ideal collection should: a) contain entries from all regions where the crop and its wild relatives grow; b) represent the range of environmental conditions within the region; and c) the size of a sample should allow an adequate number of variants to evaluate the rare alleles (Tolbert et al. 1979).

In composing the present collection, a computer search through the USDA germplasm collection identified 3,728 lines on the basis of a) spring growth habit, b) arid or semi-arid geographical origin, and c) their occurrence in salt affected areas. The remaining 1,344 entries included lines endogenous to the Arabian Pennisula and surrounding regions, and



Table 6. Estimates of the diversity index (H') for various countries, regions, species and ploidy levels, mean diversity  $(\bar{H}')$ and its standard error (SE) over salinities of 18.75  $(S_2)$  and 25.0  $dS/m$  ( $S_3$ ). Based on the mature plant stage of development

Table7. Summary of ANOVA for the diversity index (H') based on seedling survival and mature plant survival

Source of variation	Degrees of freedom	Mean square
Seedling survival		
Regions		$0.31*$
Countries within regions	12	0.09
Tolerance within countries	34	0.23
Ploidy level	3	0.13
Species within levels	6	$0.53*$
Tolerance within species	20	0.18
Mature plants survival		
Regions	4	$0.07*$
Countries within regions		0.01
Tolerance within countries	Q	0.16

\* Significant at 0.05 level

Mexican released cultivars and advanced lines. Although the collection satisfied the first two aspects of the ideal collection, an adequate sample size was not achieved in all sampled countries and or species.

Genetic diversity within a crop could be exploited to its best advantage where there are collections of germplasm with

genetic variability from wide geographic origins. Natural gene pools represent adaptive gene complexes which are of fundamental importance to the population (Marshall and Brown 1975). There are two advantages of utilising world germplasm collections to locate genes for salt tolerance. First, the gene pool is relatively large and second, interspeeific differences including wild species may serve as valuable germplasm sources.

The present germplasm collection represented 17 countries in five mostly arid and semi-arid geographical regions. Vast land resources are in arid and semi-arid regions where salinity, resulting from poor quality irrigation water and poor drainage, is a predominant factor in reducing crop productivity (Steila 1976). Although more than 5,000 entries were examined, three countries of the North African region (Algeria, Moroco and Tunisia) were poorly represented. Since the variation among countries within regions was not significant, the overall effect of these countries probably was minimal. Regional boundries are more important, they reflect the agroclimatic conditions rather than the historical and social status reflected by the political boundries (Jain et al. 1975). Mexico was represented by a large number of entries, mostly breeding lines. Since they were bred and/or selected under the general environmental conditions of the five designated regions, their presence emphasized the advantages of improving the general adaptation of the crop and enventually its tolerance to stress.

The complexity of the components of salinity largely arises from varations in plant and soil status, ion toxicity, and their interaction with environmental factors. Plant tolerance varies with the stage of development and selection at one stage is no guarantee of success at other stages (Epstein et al. 1979; Norlyn 1980). The procedure designed to classify the present collection, under controlled conditions, ensured reproducibility and precision in detecting differences among lines. Screening the collection at the seedling stage reduced the number of lines to test at other stages. Therefore, the whole-life cycle survival test involved a relatively small number of lines. Tolerance at early stages was a reliable indication of tolerance at maturity. Lines tolerant to  $S_3$  at the seedling stage were also tolerant at maturity under  $S_1$  and  $S_2$  salinities. In addition, in both early and late stages of development, the distribution of tolerance among lines and the diversity among regions and species followed similar patterns even with the restricted numbers of entries examined at maturity. Thus, the interpretation of results from early stages of development also appears to be valid for late stages.

An important outcome of the diversity analyses is to establish localised patterns of distribution for the examined trait. Therefore, the potential role of the analysis is to assist in the design of further exploration and collection efforts. Among countries of the present study, USA had the greatest proportion of tolerant lines at all stages. Most of these lines (72%) were from California and Montana. In the USA, the bulk of the salinised acreage is localised in the irrigated western arid and semi arid portion of the country. Apparantly, California has about 1.6 million hectare of saline and

saline sodic soils (Richards 1954). These areas are considered good sources of germplasm for salt tolerance. Another promising area is the North African region, especially Egypt, the country with the second greatest proportion of tolerant lines. The Nile Delta is widely known for its salt affected soils (Elgabaly 1969). Algeria, Moroco and Tunisia also should be explored for native germplasm because they are poorly represented in all world collections of wheat (Hondelmann 1979).

## **Conclusion**

The world germplasm collections of wheat, estimated at 250,000 entries, provides a broad base of genotypic variation (Moseman etal. 1979). The results of this study indicated the presence of great diversity for salt tolerance among countries, regions and species. Thus, it is suggested that the optimum strategy for future work on salt tolerance should be to concentrate on screening entries in the collection from arid and semi-arid regions. Within these regions, first priority should be given to countries with salt affected soils. Breeding material, e.g. Mexican germplasm, which has been bred under the general conditions of arid lands, deserves attention. Entries of the tetraploid species *dicoccum* seemed to have a high level of salt tolerance. Finally, the detection of salt tolerance at the seedling stage has practical merits in reducing screening efforts.

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