Pakistan J. Sci Ind. Res., Vol. 31, No. 7, July 1988

# SALT-TOLERANCE POTENTIAL OF WILD RESOURCES OF TRIBE *TRITICEAE*
## Part I. Screening of Perennial Genera

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(Received May 26, 1988, revised August 15, 1988)

The comparative salt tolerance potential of previously unscreened accessions of 19 species belonging to five different genera of the tribe *Triticeae* were tested to identify the high salt tolerant genotype to be utilized in hybridization programme. The screening was done in 254 cm x 82 cm x 23 cm cemented tanks filled with gravel and Hoagland nutrient solution. Salinity was created by mixing $NaSO_4$, $CaCl_2$, $MgCl_2$ and NaCl in the ratio of 10:5:1:4 and induced by a stepwise increase in electrical conductivity (EC) reaching of a maximum of 54 dS $m^{-1}$. Observations on plant height; no. of leaves per plant and no. of tillers per plant were recorded twice a week. Differences with respect to salinity tolerance were observed between and within the genera; *Thinopyrum* and *Leymus* were the most salt tolerant. This paper describes in detail the comparative morphology of salt tolerant and sensitive plants under saline conditions. The interspecific and intergeneric variability and the potential of tolerant species for crop improvement is also discussed.

*Key words:* Salt tolerance, *Triticeae, Thinopyrum* species, *Leymus* species, Hybridization.

## INTRODUCTION

Tribe *Triticeae*, with almost 325 species, is an excellent source of secondary gene pool for certain environmental stress conditions like salinity, alkalinity and drought [1]. Detailed salt tolerance studies conducted on various genera of this tribe have consistently shown the salt tolerance potential of the genus *Thinopyrum*, particularly of the species belonging to junceum and elongatum complex [1-7]. Other species possessing considerable salt tolerance are those belonging to the genus *Leymus* [8]. The crossability of various species of the genus *Thinopyrum* with common wheat has been established [9]. Transfer of salt tolerance from *T. ponticum* and its close allies to cultivated wheat has been successfully demonstrated [10,11].

According to the latest classification of the tribe *Triticeae* [12], the genus *Thinopyrum* consists of 20 species each represented by a number of different accessions which may differ in salt tolerance [6]. Similarly, the genus *Leymus* comprises 30 species of which *L. racemosus, (L. giganteous), L. angustus, L. sabulosus* and *L. triticoides* have so far been evaluated for salt tolerance [8].

While much variation exists for salt tolerance between and within the species [13], it is imperative to study the salt tolerance of all the available species/accessions of different genera. The inter and intraspecific variations thus obtained may be helpful in the selection of material best suitable for transfer of salt tolerance character into cultivated wheat. In the present study, 19 accessions belonging to 5 different genera of the tribe *Triticeae* were screened for salt tolerance as a part of our programme aimed at improving the crop plants for salt tolerance through wide hybridization.

## MATERIALS AND METHODS

Healthy seeds of 19 species belonging to five different genera of the tribe *Triticeae* were used in this study. The names of the species their genome and chromosome number, collection number, and origin are listed in Table 1. Ten seeds of each species were dusted with fungicide (vitavax from Ciba Giegy Switzerland) and placed in a petri plate lined with moist filtre paper. After 24 hours at room temperature ($24^{o} \pm 2$), the seeds were given cold shock for one week in a refrigerator ($4^{o}$) to break the dormancy, and again placed at room temperature. Germinated seeds were planted in 4 inch diameter plastic pots in a mixture of sand and soil (1:1) and were kept in controlled temperature room at $25^{o} \pm 2/16^{o} \pm 2$ day/night temperature, 14 hours light (35,000 lux) and 55% relative humidity A salt tolerant wheat variety LU-26-S [14] was also planted similarly to compare survival percentage, and rate of growth during vegetative stage. The wild species used are all perennial winter type grasses (except those belonging to elongatum complex viz., *T. scirpeum, T. ponticum* and *T. curvifolium*) and need vernalization to induce flowering. These three species were late in flowering compared to wheat and therefore, salt tolerance at flowering stage could not be compared.

The screening was conducted in thoroughly washed quarts (5-25 mm dia) filled in cemented tanks (254 cm long, 82 cm wide and 23 cm deep). After wetting the gravel with Hoagland nutrient solution [15] six, 2-weeks old seedlings from the pots were transferred to the tanks. After one week of growth at EC 3 dS $m^{-1}$ (control) data for plant height, number of tillers per plant and number of leaves per plant were recorded. A stepwise increase in salinity was then initiated. For this purpose the entire liquid in the tank was drained out and adjusted to desired EC by adding $NaSO_4$, $CaCl_2$, $MgCl_2$ and NaCl in the ratio of 10:5:1:4 [16]. For the first 5 weeks, EC was increased by 1 dS $m^{-1}$ every other day while during the subsequent 5 weeks a daily increase of 1 dS $m^{-1}$ was induced. To ensure sufficient nutrient supply to the plants, nutrient solution was replaced with fresh solution of required EC once a fortnight during the first 3 weeks and once a week during the subsequent study period. The experiment was carried out for 13 weeks. Observations on plant height, number of leaves per plant and number of tillers per plant were recorded twice a week. Data for plant growth reported in Tables 1-3 was recorded when a particular EC level (as specified in tables) was reached. The salt tolerant wheat variety LU-26 was harvested at EC 34 dS $m^{-1}$ (the spikes were totally sterile). Keeping this as standard, any species, irrespective of the genera, maintaining growth at conductivity above EC 34 dS $m^{-1}$ was considered salt tolerant. Most of the tolerant plants were harvested at EC 53 dS $m^{-1}$ when the symptoms of wilt and leaf browning started appearing. Remaining plants were again subjected to an other increment of salinity to give the conductivity of EC 54 dS $m^{-1}$ and harvested after 3 additional weeks.

## RESULTS

Wide variation with respect to salt tolerance was observed between and within the genera; *Thinopyrum* and *Leymus* were the most salt tolerant genera (Table 2). None of the species belonging to *Elymus, Elytrigia,* and *Pseudoroegnaria* were more tolerant than LU-26. Up to 50% of the *Leymus* species survived at EC 54 dS $m^{-1}$ and

Table 1. Description of the material used in the study.

| Genus | Species | Genome | Chromosome number | Collection number | Country of origin |
|---|---|---|---|---|---|
| *Elymus* | *Elymus canadensis* Linnaeus | SNY | 28 | Marshal Manitaba | Canada |
| *Elytrigia* | *E. repens* (L.) Nevski | SX | 28 | P1 440059 | USSR |
| *Leymus* | *L. angustus* (Trin.) Pilger | JN | 84 | P1 406461 | USSR |
| | *L. arenarius* (L.) Hochistettler | " | 56 | D 2831 | Norway |
| | *L. cinereus* (Scrib & Merr) Love | " | 28 | EPC–98 | USA |
| | *L. karelinii* (Tzvelve) D.R. Dewey | " | 56 | P1 43083 | USSR |
| | *L. racemosus* (Vahl.) | " | 28 | P1 313965 | USSR |
| | *L. sabulosus* (Bieb.) Tzvelve | " | 28 | V-Jaaska | USSR |
| *Pseudoroegnaria* | *P. stipifolia* (Czern. exNevski) Love | S | 14 | P1 440000 | USSR |
| | *P. tauri* (Boiss & Bal.) Love | " | 28 | P1 401330 | Iran |
| *Thinopyrum* | *T. bessarabicum* (Savul. & Ray SS) Love. | J–E | 14 | V. Jaaska | USSR |
| | *T. junceum* (L) Love | " | 42 | P1 277184 | France |
| | *T. curvifolium* (Lange) D.R. Dewey | " | 28 | P1 287739 | Spain |
| | *T. ponticum* (Podp.) Bark worth & D.R. Dewey. | " | 70 | P1 340066 | Turkey |
| | *T. scirpeum* (K. Presl) D.R. Dewey | " | 28 | Cauderon 720 | Italy |
| | *T. intermedium* (Host) Bark worth & D.R. Dewey | " | 42 | P1 440009 | USSR |
| | *T. podperae* (Nabelek) D.R. Dewey & Comb Nov. | " | 42 | P1 401300 | Iran |
| | *T. nodosum* (Nevski) Nevski T. caespitosum | " | 28 | Jaaska-6 | USSR |
| | *T. caespitosum* (Koch) Nevski | " | 28 | V. Jaaska-3 | USSR |

Source of Material: Dr. D.R. Dewey, USDA-ARS, Crop Research Laboratory, Utah State University, Logan, UT.

Table 2. Species survival (%) at various salinity levels (EC) of 5 defferent genera of Tribe triticeae.

| Genus | Species | Electrical conductivity (EC) $dSm^{-1}$ 3 | 5 | 10 | 15 | 20 | 27 | 34 | 41 | 48 | 53 | 54 | 54 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Leymus* | *L. cinerius* | 100 | 100 | 100 | 100 | 83 | 83 | 33 | 33 | 16 | + | + | + |
| | *L. karelinii* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | *L. arenarius* | " | " | " | " | " | " | " | " | " | " | " | 66 |
| | *L. sabulosus* | " | " | " | " | " | " | " | " | " | " | " | " |
| | *L. racemosus* | " | " | " | " | " | " | " | " | 83 | 66 | + | + |
| | *L. angustus* | " | " | " | " | " | " | " | " | " | " | + | + |
| *Thinopyrum* | *T. junceum* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 83 |
| | *T. intermedium* | " | " | " | " | 67 | 17 | + | + | + | + | + | + |
| | *T. podperae* | " | " | " | 67 | 17 | + | + | + | + | + | + | + |
| | *T. bessarabicum* | " | " | " | 100 | 100 | 100 | 100 | 100 | 83 | 66 | 33 | + |
| | *T. curvifolium* | " | " | " | 83 | 50 | 50 | 50 | 17 | + | + | + | + |
| | *T. ceaspitosum* | " | " | " | " | " | + | + | + | + | + | + | + |
| | *T. scirpeum* | " | " | " | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | *T. nodosum* | " | " | 83 | 67 | 17 | + | + | + | + | + | + | + |
| | *T. ponticum* | " | " | 100 | 100 | 100 | 100 | 83 | 53 | + | + | + | + |
| *Elymus* | *E. canadensis* | 100 | 83 | 67 | 67 | 67 | + | + | + | + | + | + | + |
| *Elytrigia* | *E. repens* | 100 | 100 | 100 | 67 | 50 | 33 | 17 | + | + | + | + | + |
| *Pseudoroegnria* | *P. stipifolia* | 100 | 100 | 67 | 17 | + | + | + | + | + | + | + | + |
| | *P. tauri* | " | " | 83 | 50 | 50 | 17 | 17 | + | + | + | + | + |
| Wheat | LU-26-S | 100 | 100 | 100 | 100 | 100 | 83 | 67 | + | + | + | + | + |

(+) Not survived.

Table 3. Plant height (% of control) of tolerant species as affected by various levels of salinity.

| Species | Electrical conductivity EC ($dS.m^{-1}$) 5 | 10 | 15 | 20 | 27 | 34 | 41 | 48 | 53 | 54 | 54 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *L. karelinii* | 6 | 13 | 33 | 62 | 64 | 66 | 61 | 55 | 50 | 46 | 20 |
| *L. sabulosus* | 3 | 14 | 42 | 92 | 100 | 105 | 93 | 78 | 67 | 43 | + |
| *L. arenarius* | 2 | 14 | 19 | 62 | 103 | 70 | 65 | 55 | 36 | 25 | + |
| *L. angustus* | 4 | 11 | 11 | 33 | 40 | 45 | 30 | 18 | 3 | + | + |
| *L. racemosus* | 2 | 13 | 37 | 76 | 86 | 92 | 67 | 50 | 50 | + | + |
| *T. scirpeum* | 1 | 3 | 16 | 24 | 18 | 18 | 10 | – 0 | – 1 | – 9 | – 12 |
| *T. junceum* | 6 | 9 | 22 | 14 | 46 | 50 | 41 | 39 | 29 | – 15 | – 16 |
| *T. bassarabicum* | – 6 | – 15 | – 15 | – 12 | – 0 | – 0 | – 3 | – 27 | – 23 | – 44 | + |
| *T. ponticum* | – 7 | – 8 | – 13 | – 12 | – 7 | – 21 | – 21 | + | + | + | + |
| LU–26–S | 39 | 132 | 155 | 216 | 244 | 214 | + | + | + | + | + |

Standard error range was 0.25-5%.

*Plant height was recorded at the time when a particular EC level was reached.

(+) Not survived.

approximately 33% were harvested at the vegetative stage (survival rate was 66-100%). Only 33% of the *Thinopyrum* species survived, of which 22% were harvested at EC 54 $dS\ m^{-1}$ (survival rate was 67-100%). Of the six species of *Leymus*, *L. karelinii* and *L. sabulosus* showed 100% survival at EC 54 $dS\ m^{-1}$. *L. sabulosus* started dying after 3 days of exposure to EC 54 $dS\ m^{-1}$ and after one week, all the plants were dead. *L. karelinii* showed 100% survival after even 3 weeks of exposure to EC 54 dS $m^{-1}$ and was harvested without any symptoms of leaf browning. Similarly, *T. junceum* and *T. scirpeum* showed survival of 83% and 100%, respectively after 3 weeks of growth at EC 54 dS $m^{-1}$. More than 50% of the species belonging to *Thinopyrum* and *Leymus* showed 100% survival at sea level salinity [(EC 46.3 dS $m^{-1}$) [17]]. The remainder of the species showed salt tolerance equal to or less than that of LU-26-S.

Plant height in all the *Leymus* species maintained an increasing trend with increasing salinity (Table 3) Salinity levels of EC 10, 15 and 20 dS $m^{-1}$ seemed to accelerate the plant growth particularly of *L. sabulosus* where plant height was almost double between EC 15 to 27 dS $m^{-1}$ compared to that observed at EC 3 dS $m^{-1}$. As salinity increased from 34 dS $m^{-1}$ a slow growth rate was observed. Consequently, the magnitude of increase in plant height was affected.

Unlike *Leymus* species, effect of increasing salinity on plant height of the *Thinopyrum* species was not uniform. *T. ponticum* and *T. bessarabicum* showed significant decrease in plant height. On the other hand, *T. junceum* and *T. scirpeum* showed significant increase in plant height after they were subjected to salinity. As in *Leymus* species, salinity level of EC 10,15 and 20 dS $m^{-1}$ also seemed to accelerate the plant growth of *T. junceum* and *T. scirpeum*. As salinity increased from EC 27 dS $m^{-1}$ a slow growth rate gradually affected the increase in plant height particularly of *T. scirpeum*. In *T. junceum*, reduction in plant height was only visible after the plants were kept longer at EC 54 dS $m^{-1}$.

Unlike plant height, number of tillers in all the tested species maintained an increasing trend with increasing salinity (Table 4). *L. karelinii, T. scirpeum* and *T. junceum* kept tillering at EC as high as 48 dS $m^{-1}$. In *L. sabulosus* and *T. bessarabicum* tiller emergence stopped after EC EC 41 dS $m^{-1}$. The maximum increase in tillering was observed in *T. scirpeum.* Generally, species of the genus *Leymus* showed poor tillering (except *L. karelinii*) compared to the genus *Thinopyrum.*

A highly significant influence of salinity on the emergence of leaves was evident from their continuous increase during the process of stepwise salinization (Table 5). As observed for tillering, *T. scirpeum* also showed maximum increase in number of leaves. Emergence of leaves in *Leymus* species particularly in *L. sabulosus, L. angustus* and *L. racemosus* was affected at higher salinity as indicated by decrease in number of healthy leaves. *Thinopyrum* species were generally healthy with dark green leaves except *T. ponticum* where emergence of leaves stopped and symptoms of wilt and leaf burning started appearing at EC 20 dS $m^{-1}$ -

## DISCUSSION

Of the 19 species tested in the present study, 6 belonged to the genus *Leymus,* 9 to *Thinopyrum,* one each to *Elymus* and *Elytrigia* and 2 belonged to the genus *Pseudoroegnaria.* Species belonging to the genera *Elymus* (SNY), *Elytrigia* (SX) and *Pseudoroegnaria* (S) showed less salt tolerance than the salt tolerant wheat cultivar LU-26 used in the present study. This may be due to the common genome "S" which possesses exceptionally high tolerance for drought rather than salt [12].

Species belonging to the genera *Leymus* and *Thinopyrum* showed considerably high salt tolerance compared to wheat cultivar LU-26. The accessions of the species

Table 4. Number of tillers (% of control) of tolerant plant species as affected by various levels of salinity.

| | Electrical conductivity (EC) dS $m^{-1}$ | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | 5 | 10 | 15 | 20 | 27 | 34 | 41 | 48 | 53 | 54 | 54 |
| *L. karelinii* | 64 | 149 | 212 | 429 | 540 | 540 | 526 | 524 | 479 | 361 | 200 |
| *L. sabulosus* | 51 | 16 | 32 | 34 | 34 | 48 | 50 | 49 | 32 | 32 | + |
| *L. arenarius* | 10 | 13 | 12 | 15 | 15 | 20 | 18 | 12 | 8 | 6 | + |
| *L. angustus* | 17 | 32 | 67 | 98 | 116 | 134 | 117 | 90 | 99 | + | + |
| *L. racemosus* | 47 | 85 | 83 | 117 | 117 | 119 | 119 | 118 | 114 | + | + |
| *T. scirpeum* | 68 | 167 | 382 | 620 | 900 | 1035 | 1137 | 1219 | 1123 | 1101 | 956 |
| *T. junceum* | 33 | 100 | 181 | 232 | 369 | 369 | 367 | 368 | 375 | 237 | 216 |
| *T. bassarabicum* | 9 | 23 | 87 | 129 | 199 | 189 | 259 | 144 | 129 | 127 | + |
| *T. ponticum* | 2 | 4 | 5 | 6 | 3 | 3 | 3 | + | + | + | + |
| LU-26-S | 3 | 4 | 4 | 4 | 5 | 4 | 3 | + | + | + | + |

Standard error range 0.25-5 %.

(+) Not survived.

Table 5. Number of leaves (% of control) of tolerant plants as affected by various levels of salinity.

| Species | Electrical conductivity (EC) dS $m^{-1}$ | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 27 | 34 | 41 | 48 | 53 | 54 | 54 |
| *L. karelinii* | 43 | 113 | 190 | 340 | 402 | 340 | 296 | 274 | 176 | 85 | + |
| *L. sabulosus* | 44 | 33 | 56 | 66 | 44 | 41 | 38 | 34 | 27 | – 24 | + |
| *L. arenarius* | 11 | 78 | 136 | 77 | 77 | 47 | 18 | 11 | 10 | 11 | + |
| *L. angustus* | 5 | 24 | 51 | 76 | 74 | 71 | 46 | 20 | – 28 | + | + |
| *L. racemosus* | 1 | 39 | 53 | 92 | 75 | 38 | 9 | 2 | – 13 | + | + |
| *T. scirpeum* | 64 | 178 | 419 | 704 | 1052 | 1153 | 1159 | 1129 | 874 | 642 | 603 |
| *T. junceum* | 38 | 78 | 133 | 211 | 277 | 250 | 242 | 177 | 124 | 81 | + |
| *T. bessarabicum* | 13 | 59 | 96 | 136 | 117 | 127 | 167 | 49 | 49 | 36 | + |
| *T. ponticum* | 21 | 82 | 162 | 40 | – 21 | – 21 | – 40 | + | + | + | + |
| LU-26-S | 93 | 142 | 187 | 237 | 135 | 34 | + | + | + | + | + |

Standard error range 0.25-5 %, (+) Not survived.

belonging to the genus *Thinopyrum* used in the present study are being screened for the first time. The pattern from lower to higher salt tolerance for these accessions is similar to that reported earlier by McGuire and Dvorak [6] except for *T. ponticum* which is comparatively less tolerant than *T. scirpeum, T. junceum* and *T. bessarabicum. T. junceum* (4X) of the *junceum* complex and *T. scirpeum* (4X) of the elongatum complex showed 100% survival after 21 days of exposure to EC 54 dS $m^{-1}$ which is less than EC 75 dS $m^{-1}$ (750 mM) used by McGuire and Dvorak [6]. However, the plant recovery in the former case is higher (100 %) than in the latter (87 %).

Among the 6 species of the genus *Leymus* only *L. racemosus* (*E. giganteus vahl,* P1 313965, WS-15-5) have been tested previously [6]. *L. racemosus, L. angustus* and *L. sabulosus* were found to be tolerant when tested at 200 mol $m^{-3}$ (EC 20 dS $m^{-1}$) NaCl solution [8]. However these authers have not mentioned the accession numbers. It is possible that the same accessions may have been included in the present study but the remaining 3 accessions have not been studied previously.

The salt level used to screen *L. racemosus, L. angustus* and *L. sabulosus* in the previous studies [8] was very low compared to the salt concentration used in our study where *L. sabulosus* has shown 100 % survival at EC 54 dS $m^{-1}$. *L. racemosus* (*E. giganteus*) have shown 87 % survival at 500 mol $m^{-3}$ (EC 50 dS $m^{-1}$) in the experiments of McGuire and Dvorak [6] whereas in the present study this accession has shown 80 % and 83 % survival at EC 50 and 53 dS $m^{-1}$, respectively. Of the remaining species *L. cinereus, L. karelinii* and *L. arenarius,* the last two showed 100% and 66 % survival, respectively after 21 days of exposure to EC 54 dS $m^{-1}$.

The genus *Thinopyrum* is known for having very high salt tolerance due to J and E genome [12]. The salt tolerance of the genus *Leymus* also has been attributed to J genome of *Thinopyrum.* The other genome N of *Psathyrostachys* may have an additive effect as it is known to impart drought tolerance and alkalinity [6]. It may be due to the combined effect of salinity and alkalinity tolerance that species of the genus *Leymus* have shown better salt tolerance compared to the genus *Thinopyrum. Leymus* species can therefore be utilized as another promising gene source for the improvement of salt tolerance in wheat.

The interspecific and intergeneric variability for salt tolerance observed in the present study have clearly emphasized a further need for collection and screening of germplasm for this particular character. The screening procedure used in this study has effectively been used to identify two *Thinopyrum* and three *Leymus* species. *Thinopyrum* species have already been hybridized with hexaploid wheat (*Triticum aestivum* L.) cultivars Chinese Spring, Filder, Fremont and Pavon. [18,19]. Of the *Leymus* species, only *L. arenarius* (2n=56) and *L. racemosus* (2n=28) have been hybridized [20,21] with *T.aestivum* (2n=42), *T. compactum* (2n=42) and *T. durum* (2n=28). *L. sabulosus* and *L. karelinii* identified as salt tolerant in the present study are now being used in a hybridization programme aimed at the improvement of salt tolerance in cereals.

*Acknowledgement.* Technical and statistical assistance of Mr. Mohammad Asghar and Mr. Ghulam Rasul Tahir, respectively, are gratefully acknowledged.

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