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Breeding for Salt-Resistance Using Transgressive Segregation in Spring Wheat

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Abstract

Hybrid populations (F_3) derived from cross between four moderate salt-resistant spring wheat (*Triticum aestivum* L.) were assessed for transgressive segregation of salt-tolerant genotypes allowing germination and emergence at 350 mM NaCl for 30 days. The achieved Selection intensity was the order of 0.24-0.27 in these segregation populations (22-25 seedlings from 9000 seeds). Assessment of transgrassive individuals (more salt tolerant) and their parents was carried out in a hydroponics experiment applying six salt concentrations of 150, 200, 225, 250, 275 and 300 Mm NaCl. Root lengths of two-week-old seedlings grown in the different concentrations were used as salt-tolerant criteria. Root length of transgressive plants were longer than parents (except Tobari 66) showing more tolerance to NaCl. It may be assumed that the character in quantitative trait based, and the parents of such hybrids may contain different genes for salt tolerance.

Keywords: Transgressive segregation; NaCl concentrations; Salt-tolerance; *Triticum aestivum*; Hybrid

Introduction

About 7% of the world's total land area is affected by salt, as is a similar percentage of its arable land [9]. The area is still increasing as a result of irrigation or land clearing [26]. Increased salt tolerance is needed for crops grown in areas at risk of salinisation. This requires new genetic sources with salt tolerance, and more efficient techniques for identifying salt-tolerant germplasm, so that new genes for tolerance can be introduced into crop cultivars [21]. Bread wheat (*Triticum aestivum* L.) is a major food crop in most of the countries of the world which suffer saline soils, and therefore increasing salinity tolerance in bread wheat is necessary. Useful variability has been shown to exist for salt tolerance in bread wheat and its progenitors. Because of its global importance as a crop, by far the greatest attention to selection and breeding for salinity tolerance has been given to *T. aestivum.*

Hybridization is a useful tool for creating genetic variation within the crop species to produce transgressive segregants. Hybridization of wheat for salt tolerance has involved crosses within species [3], between intermediately tolerant accessions seeking transgressive segregants involving salt tolerance genes exploited through complementary or interactionary

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effects, between species [3], and crosses between different genera e.g. between *T. aestivum* and some species of the genus *Agropyron* [15,28]. Hybrids have also been produced between *T. durum* and *Thinopyrum bessarabicum* [11], and/or *Aegilops* spp. [23].

Transgressive segregation is the production of plants in F_2 generation that are superior to both the parents for one or more characters. Such plants are produced by the accumulation of favorable genes from both the parents as a consequence of recombination. In this case the parents involved in hybridization must combine well with each other and preferably be genetically diverse. This way, each parent expected to contribute different plus genes which when brought together by recombination gives rise to transgressive segregation. The pedigree method as well as population approach are designed to produce transgressive segregants.

Seed germination in saline media has been used as a singular criterion for assessing salinity tolerance, or in combination with other criteria, and is a logical criterion for improving seed germination in saline conditions [16,17]. Plants peres may be more salt tolerant at germination stage, but not salt sensitive in following growth stages. Therefore, it has been proposed in some cases in which germination is more tolerant to NaCl, which the use of this criterion is not logical, since the problem of survival in later sensitive growth stages may still exist. In constant, it has been suggested that selection at germination is important and effective in species that are relatively sensitive in this stage [6]. Ability of a seed to germinate and emerge under salt stress indicates that, at least at this stage of life cycle, it has genetic potential for salt tolerance [12]. Ungar [27] showed previously that in *Hordeum jubatum* the germination stage is the most critical period determining whether it can establish itself in saline soil, while he tested *H. jubatum* for seed germination under salinity and temperature effects. Tolerance of salinity at germination and plant emergence is, however, a highly desirable trait. Norlyn and Epstein [18] showed the effectiveness of this criterion when they screened four *Triticale* lines at germination and emergence. Germination and emergence were the first indicators of salt tolerance, although there are however conflicting reports in the literature as to the relative sensitivity of germination and seedling growth to salt stress in wheat [8,29]. Salt concentration on seed germination of wheat reviled that seed germination percent, number of root, length of root and shoot significant reduced in saline Condition [28]. In genotype of wheat's that resistance to salt root length was decrease very little with addition of NaCl to the soil and index of root/shoot increased in this condition [22].

The experiments described here were carried out to examine the potential for enhancing the degree of salinity tolerance (transgressive segregants plants) in hexaploid wheat genotypes.

Material and Methods

Genetic Materials

Hybrid populations (more than 9000 seeds per each population) were produced using four moderate salt-tolerant spring wheat, *Triticum aestivum* L. as follow:

Name	*Abbreviated name*	*Origin*
1) Tobari 66	Tob	CIMMYT, Mexico
2) Siete Cerros	Sie	CIMMYT, Mexico
3) Ho2	Ho	Libya
4) Lu26.s	Lu	University of Agriculture, Faisalabad, Pakistan

These hybrid populations were Siete Cerros * Lu26.s (Sie*Lu), Tobari66 * Ho2 (Tob*Ho) and Lu26.s * Ho2 (Lu*Ho).

Screening for Salt-Tolerant Transgressive Segregants

Three screening experiments were carried out separately to screen hybrid (F_3 generation) wheat seeds for NaCl tolerance. About 9000 seeds of each of the three F_3 generations were allowed to germinate in sand treated with 350 mM NaCl in full strength Hogland nutrient solution [10] and were irrigated with saline solution every other day. Surviving individuals were selected after different times from sowing due to differences in the impact of selection on the different F_3 generations. The surviving seedlings were then transferred to another pot containing washed sand and irrigated with half nutrient solution [1] for 1-2 weeks, depending upon their conditions. They were then planted and grown to maturity in a glasshouse at 25±1°C. The seeds produced by each selected individual plant were harvested separately and their seed were increased to compare the performance of the selected lines with their parents.

Comparison between Transgressive Individuals and Their Parents

The comparison was carried out in a hydroponic experiment in a growth-room including Tobari 66 *Ho2, Lu26.s * Ho2, Siete Cerros * Lu26.s crosses and their parents.

Root and shoot lengths of two-week-old seedlings grown in the six salt concentrations, 150, 200, 225, 250, 275 and 300 mM NaCl were used for assessing salt-tolerance. These treatments were prepared in 1/10 strength nutrient solution [10]. Ten seeds per genotype were surface sterilised with 70% ethanol for 1 minute followed by 5 minutes in 5% (v/v) sodium hypochlorite solution. The seeds were then sown on 5-layer deep rafts of black alkathene beads floating on 300 cm^3 of the appropriate solution in plastic beakers. The beakers were arranged in a factorial experiment on the basis of complete randomized block design (CRBD) with three replications in a growth room at 25±1°C, relative humidity of 85%, and 27 Wm^{-2} light intensity for 16 hours daily. The beakers were placed in clear plastic chambers to minimize evaporation and to avoid the necessity to change solution during the experimental period. After two weeks of growth, root and shoot lengths of plants were measured as response indicators. The analyses of variance were carried out using GLM model in SAS programme [24].

Results and Discussion

The seed germinated after 3 weeks and some of them died due to salt, and some started to grow, some with faded leaves. Some seedlings started to shoot out the new leaves after wither having only one tiller. From the screening of the hybrid seeds Tob*Ho only 22 seedlings survived, from Lu*Ho only 25 seedlings and from Sie*Lu only 23 survived. Some of selected plants of cross Sie*Lu were corpulence. They were tall having wide leaves, large spikes and seeds.

The analyses of variance are presented in Table 1. Means of root lengths and shoot lengths of the three hybrid seedlings and their parents are shown in Table 2 and Figure 1. Root and shoot lengths of the three hybrids and their parents were significantly reduced (P<0.001) as NaCl concentration increased. Genotypes

Table 1. Mean squares from analysis of variance of root and shoot length (cm) for seedlings of three wheat hybrids (Sie*Lu, Tob*Ho, Lu*Ho) and their parents at different salt concentrations (150, 200, 225, 250, 275, 300 mM NaCl)

Source of variation	df	Root length	Shoot length
Salt Concentrations(S)	5	126.48***	3176.23***
Genotypes(G)	6	26.90***	228.47***
G*S	30	1.67***	27.94***
Block	2	2.29*	11.93*
Error	934	0.50	2.92
CV%		21	17

*** = P<0.001, *= P<0.05

Table 2. Mean root and shoot length per plants (cm) for three hybrids and their parents tested at different salt concentrations (150, 200, 225, 250 and 300 mM NaCl)

Concentrations	Genotype mean length (cm)													
	Sie*Lu		Tob*Ho		Lu*Ho		Sie		Tob		Ho		Lu	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
150 mM	2.75	10.6	2.01	11.01	3.06	10.96	1.87	9.98	2.52	11.82	1.7	9.31	1.54	8.55
	ab	ab	abc	ab	a	ab	abc	ab	abc	a	bc	ab	c	b
200 mM	2.37	8.76	1.78	9.37	2.05	7.92	1.25	7.41	1.74	9.96	1	7.25	1.03	6.80
	a	a	a	a	a	ab	b	b	a	a	b	b	b	b
225 mM	1.75	4.59	1.24	6.64	1.38	4.58	*	*	*	*	*	*	*	*
	a	a	a	a	a	a								
250 mM	1.56	3.63	0.71	5.16	0.95	3.75	*	*	*	*	*	*	*	*
	a	a	a	a	a	a								
275 mM	0.75	2.02	0.41	1.92	0.66	2.68	*	*	*	*	*	*	*	*
	a	a	a	a	a	a								
300 mM	0.5	1.59	0.2	1.11	0.42	1.89	*	*	*	*	*	*	*	*
	a	a	a	a	a	a								

R = Root, S = Shoot, * = Not emerged or not grown

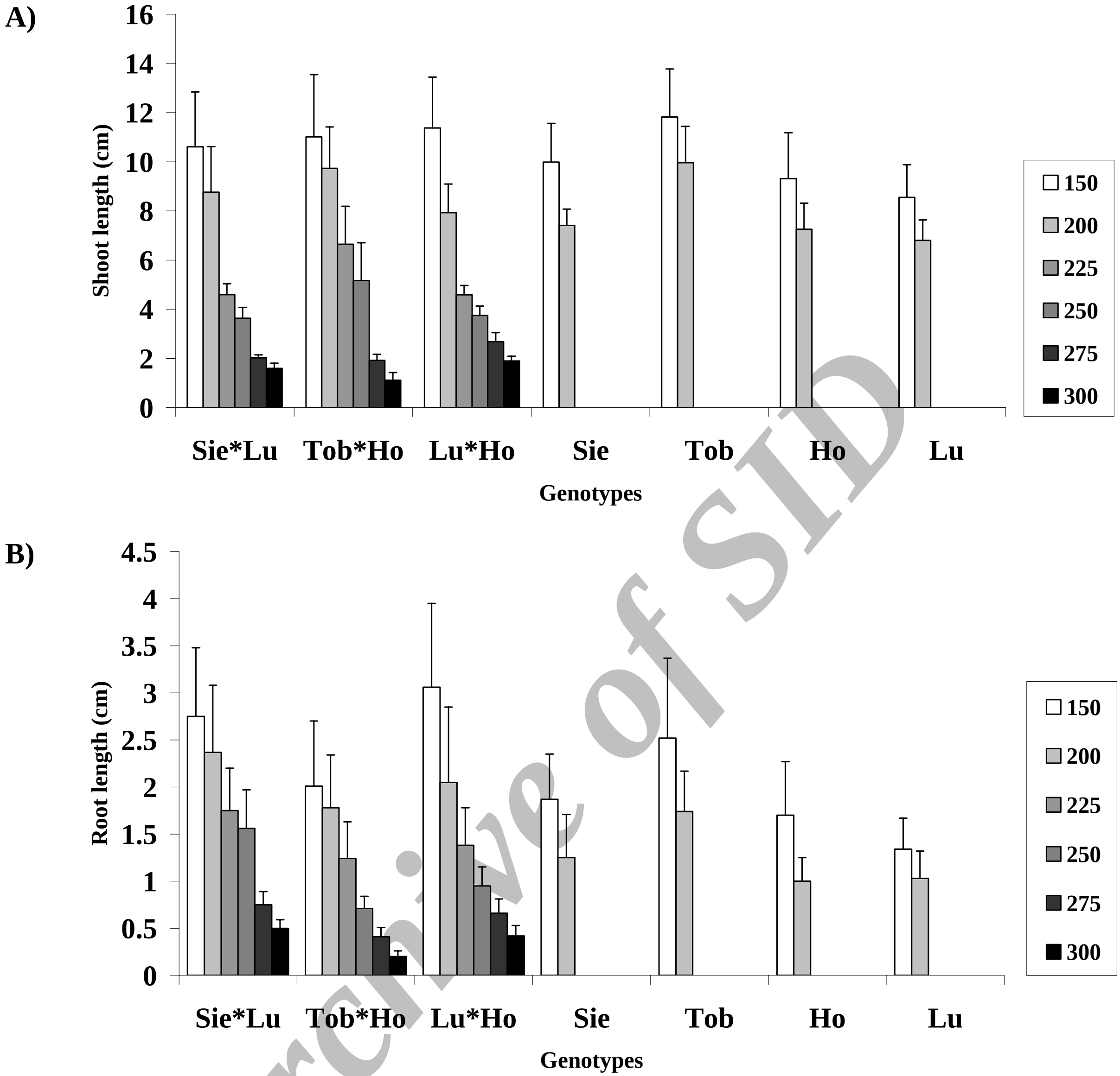


Figure 1. Mean shoot (A) and root length (B) per plants (cm) for three wheat hybrids and their parents in different salt concentrations (hybrids at 150-300Mm NaCl and their parents at 150 and 200 mM NaCl, parents could not emerge or grown in other NaCl concentrations). Bars represent SE (Standard Error).

differed significantly in root and shoot length ($P<0.001$). Interaction of salts and genotypes was significant ($P<0.001$) for root and shoot length indicating the different responses of the genotypes to increasing NaCl. The analysis of variance of the data at the 200 mM NaCl treatment, revealed significant differences between the genotypes in root and shoot length. At this level the two hybrids Sie*Lu and Lu*Ho had the longest roots, but their difference as well as the hybrid Tob* Ho and Tobari 66 were not significant. There was a significant difference in root length between the above four genotypes and others.

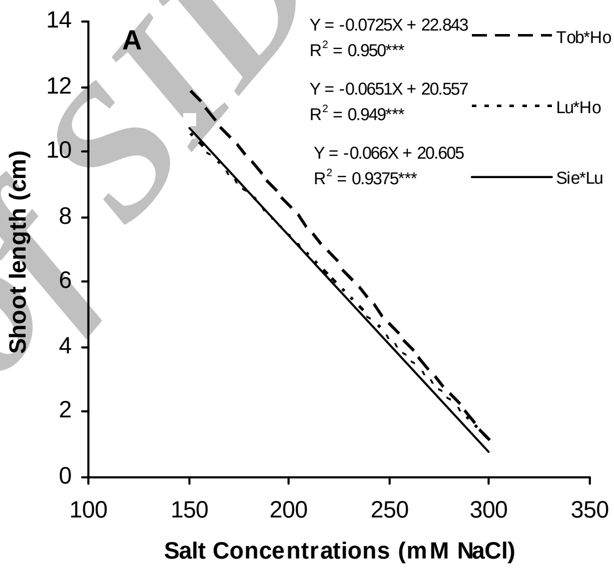
At 200 mM NaCl, Tobari 66 and Tob*Ho, and Sie*Lu had the longest shoots but the difference between them was not significant. At 300 mM NaCl the hybrids did not show any significant difference in root and shoot lengths.

Screening for more salt-resistant transgressive segregants follows the above procedure, *i.e.* screening germination 350 mM NaCl high level of salt stress. In this experiment, except for some individuals, seed germination was later than normal expected on a substratum, with a wide range. A similar effect was found by Hampson and Simpson [9] testing wheat (*T. aestivum*) germination under salt and temperature effects, where at a low osmotic potential, germination was delayed. Low osmotic potential also slows down the rate of germination, and reduces percentage of germination in both monocotyledon and dicotyledonous seeds [1,19,20].

The ability of seeds to withstand long periods of soaking in saline conditions is a characteristic of salt-tolerant species [22]. Non-tolerant wheat for example has been found to recover poorly from soaking in saline solutions [29] and increasing NaCl reduced seed germination of four forage species [5].

Screening experiment showed that after germination and emergence seedlings were affected by NaCl and some of them died at the seedling stage with increasing time of exposure to salinity. Seedling growth of wheat was more sensitive to salt stress than germination [9], and it was also reported that germination and seedling growth were affected differently by osmotic potential [29]. In the screening experiment, the frequency of selected individuals was 0.24-0.27% in the F_3 populations and all of them produced seeds. Ashraf *et al.* [4] noted that at 250 mM NaCl survival was between 0.32 to 0.80% from selection of four forage species. In another experiment using F_2 population wheat derived from parents with no known salt tolerance Ashraf and McNeilly [2] found only 0.06% survivals of seedlings selected at EC of 36 dS/m after three weeks growth. In the screening experiment, selected plants had mostly one tiller, started heading very early and produced small spikes with low numbers of seeds. These results are in agreement with the previous studies [2,4]. In salinity conditions wheat grain yield and seed number decreased [13]. Increasing salinity concentration in the rooting medium had a significant adverse effect on all yield components including number of tiller, number of grain and 1000 seed weight [4]. Early flowering in wheat is associated with early death on exposure to salt [7]. The transgressive segregants derived from intermediate salt tolerant parents, examined in hydroponic experiment, had longer roots and shoots than their parents, with exception of Tobari 66. This indicates more tolerance to NaCl, and that salinity tolerance is QTL based, and the parents of such hybrids may contain different genes for tolerance.

The regression analysis of shoot length of hybrids in NaCl concentrations (Fig. 2-A) showed that Tob*Ho hybrid had longer shoot length in control and low levels of NaCl concentration. This hybrid kept its long shoot in high level of NaCl concentration as well. That means it had an inherent ability to produce long shoot, and salinity could not reduce it severely. This hybrid also had essentially short root length and the increase of NaCl concentration reduced that even more (Fig. 2-B). Shoot and root length of Lu*Ho hybrid were 20.557 and 5.636 cm in low NaCl level and in control respectively, and the amount of reduction due to salinity is moderate for these characters. Although the Sie*Lu hybrid had a longer shoot length than Lu*Ho, but it did not have a long root length. Its root length was 4.177 cm in control. However the regression analysis of hybrids showed that Lu*Ho hybrid not only had long shoot and root length but also it kept these essential character when salinity increased.



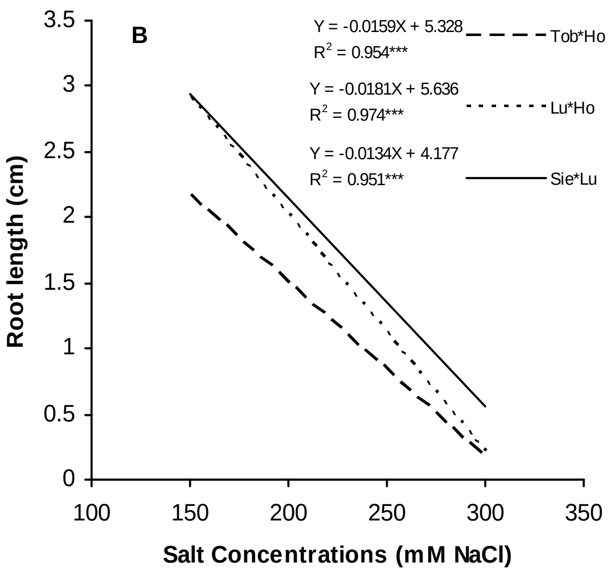


Figure 2. Liner regression correlation between increasing of salt concentration (mM NaCl) and mean shoot (A) and root length (B) per plants (cm) for three wheat hybrids (*** = P<0.001).

The procedure used in selection for more salt-resistance transgressive segregants includes selection during seed soaking in saline solution, selection for seed germination, and selection for seedling growth for the duration of the experiment. Although these only refer to the first stages of the life cycle, it can examine very large numbers of seeds, using F_2 or F_3 generation hybrids of inbreeding species, or out crossing species. From the selected material, trials such as those of Martin *et al.* [14] could be used to screen for adult salt-resistance transgressive segregants.

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