

Effect of Sodium Chloride on Germination and Growth of Hexaploid Triticale at Early Seedling Stage

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Abstract : Salt tolerance of ten cultivars of hexaploid triticale was assessed at the time of germination and at the early seedling stage in a laboratory experiment. Concentrations of the NaCl solutions employed in this experiment were 0, 50, 150, 250 and 350 mM. In none of the cultivars used in this experiment was it possible to obtain fully developed seedlings at 250 and 350 mM NaCl concentrations. Shoot growth was more sensitive to salinity than germination and root growth. It is, therefore, suggested that shoot growth is one of the most important characters for screening tests of salt tolerance in hexaploid triticale at this early stage of growth. Variation in salt tolerance among the ten cultivars was observed, with Currency, Yoreme, Welsh, and Beaver showing a higher salt tolerance in relation to germination and most of the seedling characters tested. On the other hand, Bronco was relatively sensitive to salinity in relation to both germination and seedlings characters. The varietal differences in salt tolerance were not ascribed to differences in the cytoplasm and chromosome constitution.

Key words : Germination, Hexaploid triticale, Salt tolerance, Seedling growth, Varietal difference.

六倍体ライコムギにおける発芽と苗条の生長に対する塩化ナトリウムの影響 : KARIM, M. A. ・宇都宮直樹・重永昌二 (京都大学農学部)

要 旨 : 六倍体ライコムギの発芽及び幼苗期における生長に対する塩化ナトリウムの影響を明らかにするため、10 品種を用いて室内実験を行った。用いた NaCl 水溶液の濃度は 0, 50, 150, 250, 及び 350 mM である。各濃度別に用意した濾紙敷の発芽容器に播種した後、各発芽容器にそれぞれの濃度の液を等量ずつ与え、以後、濾紙が乾燥しないように各発芽容器にそれぞれの濃度の液を 1 日 2 回等量ずつ 2 週間にわたって加えた。その結果、250 及び 350 mM 区ではどの品種も萌芽は見られたが、発芽には至らなかった。それ以下の濃度区では NaCl の濃度が高くなるほど幼苗の生長は抑制された。幼苗期の苗条の生長は、種子の萌芽及び発芽や根の伸長よりも塩類に対する高い感受性を示したので、六倍体ライコムギの生育初期における耐塩性検定の重要な指標になると考えられた。耐塩性には品種間差異が見られ、供試 10 品種の中で Currency, Yoreme, Welsh 及び Beaver は幼苗期の諸形質において高い耐塩性を示し、一方、Bronco の耐塩性は比較的低かった。ただし、このような耐塩性の品種間差異を供試品種の細胞質及び染色体構成の違いに帰することはできなかった。

キーワード : 耐塩性, 発芽, 品種間差異, 幼苗生長, 六倍体ライコムギ。

Hexaploid triticale (x *Triticosecale* Wittmack), an amphiploid between durum wheat (*Triticum turgidum* L.) and rye (*Secale cereale* L.), sometimes shows a better performance than its parental species by producing a higher yield under marginal soils and environments. Owing to such an ability, the cultivation area which has increased by one million hectares in the world^{1,13)} could extend to arid and semiarid regions where salinity is a problem for crop yield.

Crops are easily exposed to salinity immediately after planting in saline soils or in areas irrigated with brackish water. It is usually estimated that salinity results in poor plant stand due to the decrease in the germination rate of seeds and seedling survival for most of

the agricultural crops. It has also been reported that cultivars show remarkable differences in their salt tolerance⁷⁾.

Information on the salt tolerance of triticale is still insufficient, although Francois *et al.*⁵⁾ indicated that triticale is fairly tolerant to salinity. Moreover, a few experiments^{5,8)} were conducted to evaluate the response to salinity at germination and emergence, where only a few varieties/lines were employed in the experiments as experimental materials. As there are many kinds of genotypes in triticale, salt tolerance is considered to be markedly different among them.

The purpose of this study was to analyze varietal differences in the mode of response to salinity in ten cultivars of hexaploid triticale

especially at the early growth stages.

Materials and Methods

Ten cultivars of hexaploid triticale varying in their genetic background were employed (Table 1).

The experiment was conducted under laboratory conditions where the temperature fluctuated between 16 and 23°C. Forty seeds in each treatment for each cultivar were allowed to germinate on a paper towel in germination boxes. The seeds were dampened with a water solution at four different NaCl concentrations, 50, 150, 250, and 350 mM, and with distilled water as the control.

An equal amount of the respective solutions was introduced into each germination box twice a day for two weeks. The number of seeds that sprouted and germinated was counted daily. Seeds that sprouted were able to produce at least one visible plumule or radicle, whereas seeds were considered to have germinated when both shoot and root extended to more than 1.0 cm from the seeds. After final germination, the germination index (GI) was calculated by the equation :

$$GI = \frac{\text{Germination percentage in each treatment}}{\text{Germination percentage in the control}} \times 100$$

Percentage of seedlings with the first leaf and number of days to emergence of the first leaf were determined regularly. Plant height and number of roots were determined on the 14th day after seeds were sown for germination. After separation of the roots from shoot the seedlings were dried at 70°C until they reached a constant weight.

The experiment consisted of a split-plot design with four replications in which NaCl treatments were the main factor and cultivars the sub-factor. Data on the seedling characteristics for each NaCl treatment were compared with those for seedlings treated with 0 mM NaCl in each cultivar and were expressed as percentage values to the control. All the percentage data were transformed to arc sine values before statistical analysis.

Results

Sprouting

Salinity delayed sprouting. Sprouting started on the 2nd day after the seeds were sown for germination in the control, 50 and 150 mM

Table 1. Genetic background of ten cultivars of hexaploid triticale used in this experiment.

Cultivars	2n	Constitution of R-genome chromosomes	Cytoplasm
Bronco	42	2R chromosome is substituted by 2D chromosome	<i>T. turgidum</i>
Beaver	42	//	<i>T. aestivum</i>
Camel	42	2R, 4R chromosomes are substituted by 2D, 4D chromosomes	//
Welsh	42	//	//
Yoreme	42	2R chromosome is substituted by 2D chromosome	//
Beagle	42	complete	<i>T. turgidum</i>
Rosner	42	2R chromosome is substituted by 2D chromosome	//
Koala	42	//	<i>T. aestivum</i>
Tcl-18	42	complete	//
Currency	42	//	//

NaCl treatments for all the cultivars, while on the 4th day in the 250 mM treatment and on the 9th day in the 350 mM NaCl treatment. At all the concentrations, Camel took the longest time to sprout. While the percentage of sprouted seeds at both 50 and 150 mM NaCl was considerably high, ranging from 90 in Camel to 100 in Koala and Beagle, the percentage at 250 mM ranged from 60 in Camel to 71 in Currency, and at 350 mM from 5 in Rosner, Koala, and Tcl-18 to 14 in Beaver.

Germination and Germination Index

Fully developed seedlings could not be obtained in any cultivar at NaCl concentrations of 250 and 350 mM. The germination index (GI) decreased as the NaCl level increased, and a negative correlation coefficient, $r = -0.87$ (significant at $P = 0.001$), was obtained between the NaCl concentrations and GI. Varietal differences were observed in these characters (Table 2). Currency and Welsh showed the highest GI under NaCl treatment conditions. Germination indices of these two cultivars were 93 and 63, at 50 and 150 mM, respectively, followed by Koala, Tcl-18, and Beaver at 50 mM and Yoreme, Koala, and Beaver at 150 mM. Bronco showed the lowest GI at 150 mM.

Table 2. Germination percentage and germination index in ten cultivars of hexaploid triticale seeds subjected to treatments with three levels of NaCl solution.

Cultivars	NaCl		
	0 mM	50 mM	150 mM
	G	GI	GI
Bronco	95a	84cd	40c
Beaver	88ab	89abc	55ab
Camel	75c	87bc	53b
Welsh	95a	93a	63a
Yoreme	95a	82d	58ab
Beagle	85bc	82d	53b
Rosner	93a	84cd	52b
Koala	88ab	91ab	57ab
Tcl-18	95a	89abc	51b
Currency	95a	93a	63a
Avg.	90	87	55

G : Germination percentage. GI : Germination index. Avg. : Average values. Different letters within each column indicate significant difference at $P = 0.05$.

Shoot growth

a) First leaf emergence

Salinity reduced the percentage of seedlings with a fully emerged first leaf (Table 3), and delayed the first leaf emergence. A high negative correlation ($r = -0.95$, significant at $P = 0.001$) was observed between the NaCl levels and the percentage of seedlings with the first leaf. First leaf emergence started on the 6th day after the seeds were sown for germination in the control and on the 7th day at 50 mM NaCl in all the cultivars. At 150 mM, however, first leaf emergence started on the 10th day in most of the cultivars tested, though varietal differences in this character were observed. At 50 mM, Welsh, Koala, and Currency showed the highest percentage of seedlings with the first leaf, while Rosner showed the lowest percentage among the cultivars after Bronco. At 150 mM, however, the values decreased markedly for all the cultivars and no significant differences were observed in this character among the cultivars tested.

b) Plant height

The plant height of the seedlings was markedly suppressed by NaCl (Table 4). There was a high negative correlation ($r = -0.95$, significant at $P = 0.001$) between the NaCl

Table 3. Percentage of seedlings with the first leaf in ten cultivars of hexaploid triticale after seed germination at three levels of NaCl solution.

Cultivars	NaCl		
	0 mM	50 mM	150 mM
	L	%C	%C
Bronco	93a	81cd	16a
Beaver	88a	89ab	15a
Camel	75b	84bcd	13a
Welsh	93a	91a	14a
Yoreme	93a	89ab	16a
Beagle	78b	87abc	17a
Rosner	93a	78d	16a
Koala	93a	91a	14a
Tcl-18	93a	88ab	16a
Currency	93a	91a	16a
Avg.	89	87	15

L : Percentage of seedlings with 1st leaf. %C : Percentage values to the control. Avg. : Average values. Different letters within each column indicate significant difference at $P = 0.05$.

levels and plant height. At 50 and 150 mM, the plant height on the average decreased to 65 and 21% of the normal values, respectively, though varietal differences were observed. The Bronco seedlings showed the largest reduction in plant height among the cultivars tested at both levels of NaCl.

c) Shoot dry weight of seedlings

Table 5 shows that the shoot dry weight decreased with the increase in the salinity levels and the correlation coefficient between them was -0.85 (significant at $P=0.001$). At 50 mM, the dry weight reduction on an average was 50%, while it was 71% at 150 mM. At both 50 and 150 mM, Currency, Yoreme, and Welsh showed a relatively high tolerance (% to the control) to salinity while Koala and Beagle at 50 mM and Beaver, Beagle, Koala, and Rosner at 150 mM showed a tolerance similar to that of the above three cultivars, respectively. Bronco showed the lowest tolerance in relation to this character at both salinity levels.

Root growth

The number of roots per seedling was not appreciably affected by the NaCl treatments. Varietal differences in the influence on this character were observed. The root dry weight

decreased with the increase in the salinity level (Table 6). A negative and weak correlation ($r=-0.51$, significant at $P=0.001$) between the NaCl concentrations and root dry weight was observed in this experiment. At 50 mM, all the cultivars displayed a more or less similar tolerance (% to the control) except for Yoreme, but at 150 mM, Yoreme produced a high and Tcl-18 a low percentage of root mass, respectively.

Discussion

The variation in salt tolerance among the ten cultivars of hexaploid triticale was examined in this study. Although the experiment was conducted under laboratory conditions by using a NaCl solution, it is assumed that the results can be correlated with those obtained in saline soil culture as reported by Donovan and Day⁴⁾. Among the cultivars, Currency, Yoreme, Welsh, and Beaver displayed a relatively high tolerance and Bronco was more sensitive in relation to germination and most of the seedling characters tested. However, the cytoplasm and chromosome constitution of these cultivars did not affect appreciably the salt tolerance. For example, Currency, Yoreme and Welsh, all of which

Table 4. Plant height in ten cultivars of hexaploid triticale two weeks after seed germination at three levels of NaCl solution.

Cultivars	NaCl		
	0 mM	50 mM	150 mM
	H (cm)	%C	%C
Bronco	14.8abc	53e	16c
Beaver	14.5bc	68abc	21abc
Camel	11.5e	70ab	23ab
Welsh	13.8c	65abcd	26a
Yoreme	13.5d	71a	25a
Beagle	15.0abc	71a	21abc
Rosner	15.5ab	63bcd	19abc
Koala	13.8c	62cd	18bc
Tcl-18	15.8a	60de	20abc
Currency	14.3bc	66abcd	25a
Avg.	14.3	65	21

H: Plant height. %C: Percentage values to the control. Avg.: Average values. Different letters within each column indicate significant difference at $P=0.05$.

Table 5. Shoot dry weight of 100 seedlings in ten cultivars of hexaploid triticale after seed germination at three levels of NaCl solution.

Cultivars	NaCl		
	0 mM	50 mM	150 mM
	D (gm)	%C	%C
Bronco	1.13ab	38f	20c
Beaver	1.03cd	49cd	32a
Camel	0.70e	50bcd	26b
Welsh	1.00d	55ab	33a
Yoreme	0.95d	56a	33a
Beagle	1.10bc	53abcd	30ab
Rosner	1.18a	42ef	28ab
Koala	0.98d	54abc	29ab
Tcl-18	1.20a	48d	25b
Currency	1.00d	58a	33a
Avg.	1.03	50	29

D: Shoot dry weight. %C: Percentage values to the control. Avg.: Average values. Different letters within each column indicate significant difference at $P=0.05$.

have an *aestivum* cytoplasm, showed a high GI and shoot dry weight. On the contrary, Tcl-18 and Camel did not perform well in relation to these characters even though the cytoplasm and chromosome constitution of Tcl-18 and Camel were the same as those of Currency and Welsh, respectively.

The mechanism of inhibition of germination and seedling growth by NaCl which remains unclear may be related to radicle emergence due to insufficient water absorption, and mobilization of reserves from storage organs to the growing parts or may be ascribed to toxic effects on the embryo. Uhvits¹²⁾ observed in alfalfa plants that seeds absorbed an insufficient amount of water and accumulated a large amount of Cl when the osmotic pressure of the substrate was increased by salinity. As a result, the seeds emerged slowly and at higher concentrations could not germinate at all. Even after germination in saline media, the very young roots could not exclude NaCl as efficiently as mature roots¹¹⁾. Consequently, the seedlings experienced both the toxic effect of Na and Cl ions and osmotic effect associated with the high osmotic pressure in the growing media. The variations in the tolerance levels among the cultivars, however, could be

due to differences in the imbibition capacity and in the permeability of Na and Cl ions into the seeds as well as seedlings after sprouting.

The reduction rate (% values to the control) caused by NaCl was higher for parameters such as seedling height, number of seedlings with the first leaf and shoot dry weight than for germination, number of roots per seedling, and root dry weight. These findings indicate that even after adequate germination of seeds in saline soil, the seedlings may not be established well for further growth. Therefore, it is suggested that shoot growth is the most important criterion for the screening of triticale cultivars for salt tolerance at an early growth stage.

The higher tolerance at germination than at the early seedling stage in triticale cultivars is consistent with that reported in other cereal crops such as rice, barley, wheat and corn. Based on various publications, these crops display a higher salt sensitivity during emergence and early seedling growth than during germination and the later stages of growth^{2,3,6,9,10)}. Therefore, it is assumed that the salt sensitivity of hexaploid triticale is similar to that of other cereal crops throughout the growth stages. However, this assumption should be verified by further experiments including studies at the later stage of development.

Table 6. Root dry weight of 100 seedlings in ten cultivars of hexaploid triticale after seed germination at three levels of NaCl solution.

Cultivars	NaCl		
	0 mM	50 mM	150 mM
	D (gm)	%C	%C
Bronco	0.53bc	81ab	72ab
Beaver	0.58b	74ab	66abc
Camel	0.33f	76ab	70abc
Welsh	0.53bc	81ab	72ab
Yoreme	0.45cde	73b	78a
Beagle	0.43de	77ab	70abc
Rosner	0.38ef	74ab	61bc
Koala	0.45cde	78ab	67abc
Tcl-18	0.68a	74ab	56c
Currency	0.50bcd	86a	60bc
Avg.	0.49	77	67

D: Root dry weight. %C: Percentage values to the control. Avg.: Average values. Different letters within each column indicate significant difference at $P=0.05$.

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