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G.-Y. Zhong · J. Dvorak

Chromosomal control of the tolerance of gradually and suddenly imposed salt stress in the *Lophopyrum elongatum* and wheat, *Triticum aestivum* L., genomes

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Abstract The facultatively halophytic *Lophopyrum elongatum*, closely related wheat, *Triticum aestivum*, and their amphiploid tolerate salt stress better if they are gradually exposed to it than if they are suddenly stressed. *Lophopyrum elongatum* has greater tolerance of both forms of salt stress than wheat, and its genome partially confers this tolerance on their amphiploid. Chromosomal control of the tolerance of both stress regimes in the *L. elongatum* and wheat genomes was investigated with disomic and ditelosomic addition lines and disomic substitution lines of *L. elongatum* chromosomes in wheat and with wheat tetrasomics. The tolerance of the sudden salt stress is principally controlled by *L. elongatum* chromosomes 3E and 5E and less by 1E, 2E, 6E, and 7E and the tolerance of gradually imposed salt stress principally by chromosomes 3E, 4E, and 5E, and less by chromosome 1E and 7E. Ditelosomic analysis indicated that genes conferring the tolerance of sudden stress are on chromosome arms 1EL, 5ES, 5EL, 6EL, 7ES and 7EL and those controlling the gradual stress regime are on 1ES, 1EL, 5ES, 5EL, 6ES, 7ES, and 7EL. In wheat, chromosomes in homoeologous groups 1, 3, and 7 and chromosomes in homoeologous groups 1, 4, and 6 were shown to enhance the tolerance of suddenly and gradually imposed stress, respectively. The arms of chromosome 3E individually conferred tolerance to neither stress regime. Chromosome 2E and wheat chromosomes 2B and 2D reduce the tolerance of both stress regimes in a hyperploid state. In 2E this effect was associated with arm 2EL. A potential relationship between the tolerance of these stress regimes and the expression of the early-salt-induced genes is examined.

Key words Chromosomal control · Salt tolerance Wheat · *L. elongatum*

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G.-Y. Zhong (✉) · J. Dvorak
Department of Agronomy and Range Science,
University of California, Davis, CA, 95616, USA

Introduction

Lophopyrum elongatum (Host) A. Löve (2n=2x=14, genome EE) grows in salt marshes around the Mediterranean and is very tolerant of salinity (McGuire and Dvorak 1981). An octoploid amphiploid from the hybridization of *L. elongatum* with bread wheat, *Triticum aestivum* L. (2n=6x=42, genomes AABBDD) cv 'Chinese Spring', shows enhanced salt tolerance, indicating that *L. elongatum* salt tolerance is partially expressed in the genetic background of wheat (Dvorak and Ross 1986; Dvorak et al. 1988; Omielan et al. 1991).

Lophopyrum elongatum and wheat are partial "ion excluders" when stressed with salt (Storey et al. 1985; Schachtman et al. 1989; Omielan et al. 1991). Because ion exclusion likely requires the synthesis of compensatory osmolytes, it seems reasonable to expect that physiological mechanisms that enable ion excluders to function under salt stress are energetically expensive and would be activated only under stress. In that case, a gradual exposure to salt stress would be expected to have less severe consequences on plant growth than a sudden exposure to the same level of stress, since it would give plants time to alter gene expression for growth under salt stress and acclimate. This assumption has been found to be true in sorghum (Amzallag et al. 1990), wheat, and the amphiploid between wheat and *L. elongatum* (Dvorak et al. 1991).

Direct evidene showing that wheat and *L. elongatum* respond to salt stress by altering gene expression comes from studies on the accumulation of mRNAs after an exposure to salt stress (Gulick and Dvorak 1987, 1992). Soon after an exposure to salt stress, both *L. elongatum* and wheat roots begin to accumulate mRNAs of a group of coordinately regulated genes of the *Esi* (Early-salt-induced) gene system. Accumulation of these mRNAs is biphasic. The first phase occurs within several hours after an exposure to salt and mRNA levels peak within 6–12 hours (Gulick and Dvorak 1992; Galvez et al. 1993). This phase is primarily a response to salt shock and is higher in *L. elongatum* and the amphiploid than in wheat (Galvez et al. 1993).

If the accumulation of these mRNAs in the first phase reflects a genetic response to sudden exposure to salt, *L. elongatum* and the amphiploid should tolerate a sudden exposure to salt better than wheat.

In this study, the rates of leaf elongation in *L. elongatum,* wheat, and their amphiploid were used as the means of quantitatively comparing the tolerance of gradually and suddenly imposed salt stress by these three genotypes. Wheat-*L. elongatum* disomic and ditelosomic addition lines and disomic substitution lines were employed to investigate the chromosomal control of the tolerance of both stress regimes in the *L. elongatum* genome and to determine if the chromosomal control coincides with the location of the *Esi* genes (Dubcovsky et al. 1994). A similar investigation was carried out with wheat tetrasomics to determine if increased doses of specific wheat chromosomes have similar effects on the tolerance of early salt stress as additions of homoeologous *L. elongatum* chromosomes into wheat.

Materials and methods

Plant materials

Triticum aestivum cv 'Chinese Spring' was provided by E. R. Sears, University of Missouri, Columbia. The amphiploid 'Chinese Spring'× *L. elongatum* was developed by A. Mochizuki and B. C. Jenkins (unpublished) and was supplied by D.R. Knott, University of Saskatchewan, Saskatoon. *Lophopyrum elongatum* was supplied by L. E. Evans, University of Manitoba, Winnipeg, Canada. The three stocks were single-plant progenies inbred for more than five generations in the greenhouse. Further used were disomic addition lines (henceforth DA) of each of the seven *L. elongatum* chromosomes in the genetic background of 'Chinese Spring' wheat (Dvorak 1980; Dvorak and Chen 1984; Dvorak et al. 1984; Tuleen and Hart 1988), ditelosomic addition lines (henceforth DTA) for 13 of the 14 chromosome arms of *L. elongatum* in 'Chinese Spring' (Dvorak 1979; Hart and Tuleen 1983), and disomic substitution lines (henceforth DS) of each of the *L. elongatum* chromosomes for its 'Chinese Spring' homoeologous chromosomes (Dvorak 1980; Dvorak and Chen 1984; Tuleen and Hart 1988). The *L. elongatum* chromosomes are designated according to their homoeology with wheat chromosomes followed by the letter E. The replaced wheat chromosomes in DS lines are specified in parentheses. 'Chinese Spring' tetrasomics (henceforth Tetra) were supplied by E. R. Sears (Sears 1954).

Experimental design

Seeds were surface sterilized with diluted commercial bleach 1:1 (v/v) for 15 min, rinsed in distilled water, and germinated in a vertical position between sheets of blotting paper in direct sunlight. When the roots of the seedlings were about 10 cm long the seedlings were transferred into tanks, each holding 150 l of nutrient solution and 120 seedlings. In the experiments in which comparisons were made between *L. elongatum*, 'Chinese Spring', and their amphiploid, the solution was 0.5×Hoagland (Epstein, 1972) modified by increasing the $Ca(NO_3)_2$ concentration to 23 mM. The solution was adjusted to 28 m*M* FeEDTA and a pH of 5.2–5.4 and the tanks were aerated. Another nutrient solution has been recently reported by Huang et al. (1992), and since we believe that this nutrient solution is superior to one we originally used, this nutrient solution was employed in comparisons among DA lines, DTA lines, DS lines, and wheat tetrasomics. When the plants began to tiller, the solution was adjusted to the desired salinity either in 50 m*M* increments every third day or in a single step. In each experiment, one or more tanks received no salt (control). Daily leaf elongation was determined by marking emerging leaves and measuring the distance which the marks moved from a fixed point during 24 h. Leaf elongation of the plants in the treatment tanks was expressed in millimeters and in percentages of the mean leaf elongation of the unstressed plants of the same genotype in the control tanks.

A total of seven experiments are reported. Comparison between these experiments should be made with caution because they were conducted in different environments. For that reason, the results of these experiments were analyzed separately.

The tolerance of gradual and sudden exposure to 250 m*M* NaCl by *L. elongatum*, 'Chinese Spring', and their amphiploid was investigated in experiment 1. The experiment consisted of two treatment tanks and one control tank in a greenhouse. Each tank contained three blocks of 5–12 plants of each genotype. The entire experiment was replicated in time, March and June. The experiment was statistically analyzed with analysis of variance for a split plot design in which the genotypes were nested within the treatments over two planting dates.

Lophopyrum elongatum was gradually or suddenly exposed to 450 m*M* NaCl in greenhouse experiment 2. The experiment consisted of two treatment tanks and one control tank each containing 36 *L. elongatum* plants. Daily leaf elongation of individual plants, expressed as percentage of the control, were variables in the *t*-test.

The tolerance of sudden and gradual exposure to 250 m*M* NaCl by 'Chinese Spring', the 'Chinese Spring'× *L. elongatum* amphiploid, DA lines, DS lines, and 'Chinese Spring' tetrasomics was investigated in experiments 3 and 4, respectively. Experiment 3 consisted of seven treatment tanks with salt and one control tank, whereas experiment 4 consisted of seven treatment tanks and three control tanks. Because of the size of these experiments, they were conducted outdoors to minimize variation among the tanks that would occur in a greenhouse due to temperature and humidity gradients. The size of these experiments also precluded conducting them simultaneously. Three or four plants of each genotype were planted per tank in a completely randomized design. When plants began to tiller the solution was either adjusted to 250 m*M* NaCl in a single step (sudden exposure) or in 50-m*M* increments every third day (gradual exposure).

The tolerance of sudden exposure to 250 m*M* NaCl by DTA lines was compared with that of 'Chinese Spring' in experiments 5 and 6 and the tolerance of gradual exposure to 250 m*M* NaCl in experiment 7. Experiments 5 and 6 were conducted outdoors whereas experiment 7 was conducted in the greenhouse. Experiment 5 consisted of four salt treated tanks and one control tank. Six plants of each genotype were planted per tank. Experiment 6 consisted of four treatment and two control tanks. Experiment 7 consisted of three treatment tanks and one control tank.

Leaf elongation was determined from days 3 to 6 after reaching 250 m*M* NaCl in experiments 1 and 2 and from days 3 to 5 in experiments 3 to 7. In the experiments in which salinity was gradually imposed, leaf elongation was also measured from days 21 to 23.

Because the numbers of tanks used for control and salt treatments were unbalanced and we were at that point mainly interested in the chromosomal control of salt tolerance rather than comparison of the sudden and gradual stress regimes, the analysis of variance and mean comparisons in experiments 3–7 were carried out separately for the untreated control tanks and treatment tanks. A completely randomized design was used when only one tank was used as a control, and a randomized complete block design was used only when two or more control tanks were used. The treatment tanks were analyzed as a randomized complete block design. The significance of variation among the lines was tested with LSD.

For those lines for which the rates of leaf elongation of the unstressed control plants differed significantly from 'Chinese Spring', the rates of leaf elongation of stress plants expressed as percentage of the unstressed control were computed and are reported. Mean daily leaf elongations expressed as percentage of the unstressed controls were log transformed and analyzed with analysis of variance and LSD. In those cases in which the mean daily leaf elongation expressed as percentage of unstressed control was inconsistent, in terms of statistical significance, with that expressed in millimeters, the weight was placed on the significance, or lack of it, of the mean daily leaf elongation expressed as percentage of unstressed control.

Results

Sudden versus gradual exposure to salt stress in *L. elongatum*, wheat, and their amphiploid

Daily leaf elongation rates in *L. elongatum*, wheat, and their amphiploid after sudden and gradual exposure to stress with 250 m*M* NaCl were investigated for 20 days (experiment 1). Leaf elongation immediately decreased in all three genotypes after sudden exposure to 250 m*M* NaCl. In 'Chinese Spring' and the amphiploid leaf elongation then gradually declined to day 6, and by day 8 most plants were dead. This was not true for *L. elongatum* in which growth recovered to about 40% of the unstressed control and continued at that rate for the entire period of 20 days.

The rates of leaf elongation in 'Chinese Spring' and the amphiploid declined after gradual exposure to 250 m*M* NaCl, but less rapidly than when these genotypes were exposed to NaCl suddenly. A portion of the plants of both genotypes died, but some grew throughout the entire period. The rate of leaf elongation in *L. elongatum* declined at first, but after several days it plateaued at a rate similar to that of plants stressed suddenly. Daily rates of leaf elongation of the three genotypes under these two stress regimes have been graphed in Fig. 2 of Dvorak et al. (1991) and will not be shown here.

Mean rates of leaf elongation from day 3 to day 6 were used as variables in the analysis of variance to investigate the statistical significance of the differences among the three genotypes. In all three genotypes leaf elongation was significantly greater in plants stressed gradually than in those stressed suddenly. The rates of leaf elongation in *L. elongatum* were significantly greater than those in the amphiploid at both stress regimes, and the rates in the amphiploid were significantly greater than those in 'Chinese Spring' at both stress regimes (Table 1).

Lophopyrum elongatum was stressed with 450 m*M* NaCl applied either suddenly or gradually in experiment 2 (data not shown). While the plants that were gradually exposed to 450 m*M* NaCl survived and grew at a more-or-less constant rate of about 20% of the unstressed control, those exposed suddenly stopped growth and died within 2 days. The daily rates of leaf elongation in gradually stressed plants were significantly different (*t*-test) from those of plants stressed suddenly (days 1 and 2), or from zero (days 3–14). This showed that a gradual exposure to salt stress is as beneficial to the survival of *L. elongatum* plants at a high stress level as is the gradual exposure to wheat at a lower stress level.

The tolerance of sudden exposure to salt stress by DA and DS lines and wheat tetrasomics

The chromosomal control of the enhanced tolerance of sudden exposure to salt stress conferred on the amphiploid by the *L. elongatum* genome was investigated by comparing the rates of leaf elongation in 'Chinese Spring' × *L. elongatum* disomic addition lines and disomic substitution lines with the rates found in salt-stressed 'Chinese Spring' (experiment 3).

Table 1 Mean daily leaf elongation in millimeters and percentage of unstressed controls from days 3 to 6 after exposure of *L. elongatum*, 'Chinese Spring' (CS), and their amphiploid to 250 m*M* NaCl either suddenly or gradually in 50 m*M* steps

Line	Mean daily leaf elongation[a]				
	Control	Sudden exposure		Gradual exposure	
	mm	mm	% control	mm	% control
L. elongatum	22.3	4.29	19.2	12.1a	54.7
Amphiploid	49.3b	1.89	3.8	12.0a	24.3
Chinese Spring	44.9b	0.61	1.4	7.4	16.5

[a] Comparable means in rows and columns are significantly different from each other except for those followed by a common letter

There was no difference in the rate of leaf elongation between the unstressed amphiploid and unstressed 'Chinese Spring' in the control tank. The amphiploid showed a significantly greater rate of leaf elongation than 'Chinese Spring' when suddenly stressed with 250 m*M* NaCl (Table 2).

The only *L. elongatum* chromosome which did not affect the tolerance of this salt stress regime was 4E (Table 2). Added or substituted chromosomes 1E, 3E, 5E, 6E and 7E increased the rate of leaf elongation relative to that shown by 'Chinese Spring' (Table 2), and added chromosome 2E decreased the rate of leaf elongation. However, none of the DS lines involving chromosome 2E showed a reduction in the rate of leaf elongation; DS2E(2B) showed a significant increase (Table 2).

Wheat tetrasomics 1A, 3B, 3D, and 7A showed greater rates of leaf elongation than 'Chinese Spring' while the leaf elongation rates in tetrasomics 2B, 2D, and 6D were significantly lower than that of 'Chinese Spring' (Table 2).

The tolerance of gradual exposure to salt stress by DA and DS lines and wheat tetrasomics

The same lines as in experiment 3 were stressed gradually (experiment 4) and the rates of leaf elongation were determined from days 3 to 5 and days 21 to 24 after the salinity of the solution reached 250 m*M* NaCl (Table 2).

Disomic addition or DS lines with *L. elongatum* chromosomes 3E, 4E, and 5E showed increased rates of leaf elongation relative to 'Chinese Spring' from days 3 to 5 as well as from days 21 to 24. Disomic addition line 2E showed a decreased rate of leaf elongation relative to 'Chinese Spring' from days 3 to 5, but DS2E(2B) showed increased rates from days 3 to 5.

Wheat tetrasomics 1A, 4D, and 6A showed greater rates of leaf elongation than 'Chinese Spring' whereas tetrasomics 2B and 2D showed lower rates at days 3 to 5. No tetrasomic differed significantly from 'Chinese Spring' at days 21–24 (Table 2).

Table 2 Mean daily leaf elongation in millimeters or percentage of unstressed controls of wheat-*L. elongatum* disomic addition lines (DA), disomic substitution lines (DS), 'Chinese Spring' tetrasomics (Tetra), 'Chinese Spring', and the amphiploid 'Chinese Spring'× *L. elongatum* from days 3 to 5 after sudden exposure to 250 m*M* NaCl (experiment 3) and from days 3 to 5 and 21 to 23 after gradual exposure to 250 m*M* NaCl (experiment 4)

Line	Mean daily leaf elongation[a]						
	Control	Sudden exposure		Control	Gradual exposure		
		Days 3–5			Days 3–5		Days 21–23
	mm	mm	% control	mm	mm	% control	mm
Chinese Spring	12.2	3.7	30.3[b]	10.5	2.1	20.0	0.3
Amphiploid	11.2	5.3$^+$		11.1	5.7$^+$		6.5$^+$
DA1E	12.5	4.6$^+$		8.8$^-$	2.2	25.0	0.0
DS1E(1A)	12.7	3.8		9.5	3.0		0.5
DS1E(1B)	13.0	2.8$^-$		9.6	2.5		0.4
DS1E(1D)	13.7	4.3		11.0	2.9		0.5
Tetra1A	12.2	4.4$^+$		9.0$^-$	3.0	33.3$^+$	0.7
Tetra1B	13.2	3.9		8.5$^-$	2.0	23.5	0.0
Tetra1D	10.2	4.0		8.3$^-$	2.3	27.0	0.3
DA2E	11.7	1.5$^-$		9.3$^-$	0.6$^-$	6.5$^-$	0.0
DS2E(2A)	13.8	3.7		8.8$^-$	1.8	20.4	0.0
DS2E(2B)	12.6	5.2$^+$		8.0$^-$	2.7	33.8$^+$	0.2
DS2E(2D)	15.3$^+$	4.1	26.8	10.4	2.9		0.3
Tetra2A	12.7	3.7		9.6	2.2		0.3
Tetra2B	12.3	2.5$^-$		8.2$^-$	1.0$^-$	12.2	0.2
Tetra2D	12.1	2.1$^-$		9.6	0.6$^-$		0.0
DA3E	12.3	5.8$^+$		9.0	3.1$^+$		0.7
DS3E(3A)	15.0$^+$	5.2$^+$	34.7	10.1	3.9$^+$		2.2$^+$
DS3E(3B)	13.5	5.2$^+$		8.3$^-$	2.5	30.1	1.3$^+$
DS3E(3D)	13.0	4.1		9.1$^-$	3.7$^+$	40.7$^+$	2.5$^+$
Tetra3B	13.3	4.9$^+$		10.1	2.6		0.0
Tetra3D	14.3	4.7$^+$		9.3$^-$	1.6	17.2	0.0
DA4E	13.2	4.0		8.8-	3.3$^+$	37.5$^+$	0.7
DS4E(4A)	9.0$^-$	3.1	34.4	6.7$^-$	3.5$^+$	52.2$^+$	1.3
DS4E(4D)	13.4	3.6		8.9$^-$	3.2$^+$	36.0$^+$	1.5
Tetra4D	13.4	3.5		10.1	3.4$^+$		1.1
DA5E	10.8	4.5$^+$		10.0	4.3$^+$		2.3$^+$
DS5E(5B)	13.3	5.4$^+$		9.2$^-$	1.9	20.7	0.0
DS5E(5D)	11.9	4.2		10.6	2.3		0.2
Tetra5D	12.8	4.4		9.0$^-$	2.3	25.5	0.0
DA6E	14.0	3.4		8.5$^-$	1.9	22.4	0.0
DS6E(6A)	14.2	4.6$^+$		10.6	2.5		0.1
DS6E(6B)	12.4	5.5$^+$		9.5	2.4		0.0
DS6E(6D)	13.3	4.9$^+$		9.7	2.7		0.4
Tetra6A	12.9	3.4		9.8	3.7$^+$		0.5
Tetra6B	14.7$^+$	4.0	27.2	10.1	3.0		0.8
Tetra6D	13.2	2.4$^-$		9.1$^-$	2.0	22.0	0.1
DA7E	14.2	4.8$^+$		9.3$^-$	2.7	29.0	0.3
DS7E(7A)	12.7	4.7$^+$		9.8	2.7		0.5
DS7E(7B)	13.0	5.2$^+$		10.1	2.6		0.5
DS7E(7D)	12.9	3.8		8.8$^-$	2.7	30.7	0.4
Tetra7A	14.2	5.3$^+$		8.2$^-$	2.0	24.4	0.0
Tetra7D	13.4	4.4		9.5	2.5		0.6

[a] + and – signs indicate means in columns significantly higher and lower than Chinese Spring, respectively, at the 5% probability level

[b] Leaf elongation in% of unstressed control is shown only for those lines for which unstressed controls differed significantly from 'Chinese Spring'

The tolerance of sudden exposure to salt by DTA lines

The arm locations of *L. elongatum* genes affecting the tolerance of sudden exposure to salt stress were investigated in experiments 5 and 6. Experiment 5 was conducted in June, 1993. The effect of salt stress was exaggerated by temperatures above 30°C and wind. Only stressed DTA lines 1EL and 5EL showed significantly greater rates of leaf elongation than 'Chinese Spring' (only data which were significantly different from 'Chinese Spring' are shown in Table 3). The experiment was repeated in October, 1993 (experiment 6) to reinvestigate these 2 DTA lines and those DTA lines for which the complete chromosomes showed rates of leaf elongation significantly different from that of 'Chinese Spring' in experiment 3. Disomic addition lines were included for comparison with DTA lines.

Table 3 Mean daily leaf elongation in millimeters and percentage of unstressed controls of wheat-*L. elongatum* ditelosomic addition lines (DTA), disomic addition lines (DA), 'Chinese Spring', and the amphiploid 'Chinese Spring'× *L. elongatum* from days 3 to 5 after sudden exposure to 250 m*M* NaCl (experiments 5 and 6) and from days 3 to 5 and 21 to 23 after gradual exposure to 250 m*M* NaCl (experiment 7)

Line	Mean daily leaf elongation[a]						
	Control	Sudden exposure		Control	Gradual exposure		
		Days 3–5			Days 3–5		Days 21–2
	mm	mm	% control	mm	mm	% control	mm
Chinese Spring	20.7	1.2	5.8[c]	22.8	14.1	62.2	0.8
Amphiploid	20.6	3.5$^+$		24.6	16.6$^+$		12.2$^+$
Chinese Spring (5)[b]	27.8	1.9	6.8	–	–	–	–
Amphiploid (5)	24.2	6.1$^+$		–	–	–	–
DTA1ES (5)	30.2	2.3		–	–	–	–
DTA1EL (5)	25.9	3.1$^+$		–	–	–	–
DA1E	–	–		21.3	14.3		4.4$^+$
DTA1ES	–	–		24.4	14.8		5.3$^+$
DTA1EL	20.5	2.0$^+$		22.5	14.9		4.3$^+$
DA2E	20.6	1.2		22.1	14.4		0.9
DTA2ES	21.9	1.2		22.1	14.9		5.9$^+$
DTA2EL	21.2	1.7		24.9$^+$	12.8	51.4$^-$	0.0
DA3E	23.0$^+$	4.4$^+$	19.1$^+$	23.1	13.4		1.1
DTA3ES	19.2$^-$	1.5	7.8	22.6	12.5		0.9
DTA3EL	20.9	1.6		20.6$^-$	13.6	66.0	0.7
DA4E				21.9	12.6		5.2$^+$
DTA4EL (5)	23.4$^-$	1.9	8.1	22.9	12.9		2.2
DA5E	19.6	5.1$^+$		22.6	14.2		7.9$^+$
DTA5ES	20.3	1.9$^+$		23.7	14.6		4.2$^+$
DTS5EL	22.6	2.4$^+$		26.4$^+$	16.8$^+$	63.6	7.0$^+$
DA6E	20.2	1.4		23.3	13.8		2.8
DTA6ES	19.3	0.9		24.1	14.4		5.0$^+$
DTA6EL	19.6	2.9$^+$		23.1	13.1		2.4
DA7E	23.3	2.5		25.7$^+$	16.3$^+$	63.4	0.5
DTA7ES(α)	23.6	2.2		22.8	16.1$^+$		3.1$^+$
DTS7EL(β)	21.6	2.0		24.3	15.7$^+$		2.5$^+$

[a] + and – signs indicate means in columns significantly higher and lower than Chinese Spring, respectively, at the 5% probability level
[b] (5): data from experiment 5
[c] Leaf elongation in % of unstressed controls is shown only for those lines for which unstressed controls differed significantly from 'Chinese Spring'

Compared to experiment 3, all lines showed lower rates of leaf elongation in salt-treated tanks, indicating greater stress due to uncontrollable environmental factors in experiment 6. As a result, DA lines that were marginally significantly different in the rates of leaf elongation from 'Chinese Spring' in experiment 3, DA1E, DA2E, and DA7E, did not significantly differ in this experiment (compare Tables 2 and 3). The inability to declare minor significant differences from 'Chinese Spring' significant when the rate of leaf elongation of 'Chinese Spring' and many other lines was close to zero, combined with other genotype×environment interactions apparent in comparisons of experiments 3 and 6 and 4 and 7 (see below), shows that inconsistencies in the statistical significance of minor effects must be expected when experiments are conducted in different environments.

In spite of problems with comparing different experiments, DTA lines 1EL and 5EL again showed significantly greater rates of leaf elongation than 'Chinese Spring' in experiment 6. The 3E ditelosomic addition lines did not significantly differ from 'Chinese Spring', although DA3E showed a significantly greater rate of leaf elongation than 'Chinese Spring' in the same tanks. Ditelosomic addition line 5ES, in addition to the already mentioned DTA5EL, showed a greater rate of leaf elongation than 'Chinese Spring'; the rates for both DTA lines were significantly lower than that of DA5E. Ditelosomic addition line 6EL showed a significantly greater rate of leaf elongation than 'Chinese Spring'; DA line with the complete chromosome 6E did not significantly differ from 'Chinese Spring'.

The tolerance of gradual exposure to salt stress by DTA lines

The arm locations of *L. elongatum* genes affecting the tolerance of gradual exposure to salt stress was investigated in experiment 7. The rate of leaf elongation of DA2E did not significantly differ from that of 'Chinese Spring', but the rate of DTA2EL was significantly lower when expressed as percentage of the unstressed control than that of 'Chinese Spring' from days 3 to 5 (Table 3). This sug-

Table 4 Mean daily leaf elongation in millimeters and percentage of unstressed controls in 'Chinese Spring', the amphiploid 'Chinese Spring'×*L. elongatum*, and per homoeologous group and per genome computed from the means of wheat-*L. elongatum* disomic substitution lines (DS), disomic addition lines (DA), and wheat tetrasomics (Tetra) from days 3 to 5 after sudden stress with 250 m*M* NaCl (experiments 3) and from days 3 to 5 and 21 to 23 after gradual exposure to 250 m*M* NaCl (experiment 4)

Line	Mean daily leaf elongation[a]					
	Control	Sudden exposure	Control	Gradual exposure		
		Days 3–5		Days 3–5		Days 21–23
	mm	mm	mm	mm	% control	mm
Chinese Spring	12.2	3.7	10.5	2.1	20.0[b]	0.3
Amphiploid	11.2	5.3$^+$	11.1	5.7$^+$		6.5$^+$
DS1	13.0	3.8	9.8	2.6		0.4
DS2	13.8	4.0	9.2$^-$	2.1	22.8	0.1
DS3	13.5	5.1$^+$	9.1$^-$	3.3$^+$	36.3$^+$	1.6$^+$
DS4	11.9	3.6	8.2$^-$	3.4$^+$	41.5$^+$	1.5$^+$
DS5	12.0	4.7$^+$	10.0	2.9$^+$		0.9
DS6	13.5	4.6$^+$	9.5$^-$	2.4	25.3	0.1
DS7	13.1	4.6$^+$	9.5$^-$	2.7	28.1	0.4
Tetra1	11.9	4.1	8.7$^-$	2.4	28.1	0.3
Tetra2	12.4	2.9$^-$	9.2$^-$	1.3$^-$	14.1	0.1
Tetra3	13.8	4.8$^+$	9.7	2.1		0.0
Tetra4	13.4	3.5	10.1	3.4$^+$		1.1
Tetra5	12.8	4.4	9.0$^-$	2.3	25.6	0.0
Tetra6	13.6	3.3	9.7	2.9$^+$		0.5
Tetra7	13.8	4.8$^+$	8.9$^-$	2.2	24.7	0.3
DA genome E	12.7	4.3$^+$	9.1$^-$	2.7	29.7	0.8
DS genome A	12.7	4.2	9.3$^-$	2.9$^+$	31.2$^+$	0.7
DS genome B	13.0	5.0$^+$	9.1$^-$	2.4	26.4	0.4
DS genome D	13.4	4.1	9.8	2.9$^+$		0.8
Tetra genome A	13.0	4.2	9.2$^-$	2.7	29.3	0.4
Tetra genome B	13.4	3.8	9.2$^-$	2.2	23.9	0.2
Tetra genome D	12.8	3.7	9.3$^-$	2.1	22.6	0.3

[a] + and – signs indicate means in columns significantly higher and lower than Chinese Spring, respectively, at the 5% probability level
[b] Leaf elongation in % of unstressed control is shown only for those lines for which unstressed controls differed significantly from 'Chinese Spring'

gests that the decrease in the leaf elongation rate of DA2E documented earlier (Table 2) is controlled by the long arm. Ditelosomic addition lines 5EL, 7ES and 7EL grew at a significantly greater rate than 'Chinese Spring' from days 3 to 5. Both 7E DTA lines showed greater rates of leaf elongation than 'Chinese Spring'. Disomic addition line DA7E with the complete chromosome 7E differed significantly from 'Chinese Spring' only in the terms of growth in millimeters. When the leaf elongation rates of DA7E and CS were expressed as percentage of the unstressed controls they did not significantly differ (Table 3).

The picture changed dramatically 3 weeks later. Most 'Chinese Spring' plants died at that point, and many DTA lines showed greater rates of leaf elongation than 'Chinese Spring' (Table 3). These data are reported only in terms of growth rates in millimeters. The plants in the untreated tank were much more advanced than the stressed plants and expressing the growth rates in terms of percentage of unstressed control would have been of dubious value. The rates were greater than those of 'Chinese Spring' for ditelosomics for both arms of chromosomes 1E, 5E, and 7E, and for those for the short arms of chromosomes 2E and 6E. Neither of the 3E DTA lines showed enhanced salt stress tolerance. Ditelosomic addition line 4EL showed a significantly lower rate of leaf elongation than DA4E and did not differ from 'Chinese Spring'.

Comparison among homoeologous groups and genomes

The rates of leaf elongation of the 3 disomic substitution lines within each homoeologous group were averaged to obtain an idea about the overall effect of a *L. elongatum* chromosome on the tolerance of each of the two stress regimes. Only homoeologous groups with the most consistent effects were expected to deviate significantly from 'Chinese Spring'. The tolerance of sudden salt stress was enhanced by chromosomes 3E, 5E, 6E, and 7E; chromosomes 3E and 5E appeared to play the most significant role. The same analysis showed a significant enhancement of the tolerance of gradually imposed salt stress by chromosomes 3E, 4E, and 5E; chromosomes 3E and 4E played the most significant role. All enhancements of the tolerance of either stress regime in the DS lines were significantly lower than those observed in the amphiploid.

The same analysis with wheat tetrasomics indicated that homoeologous groups 3 and 7 play a role in the tolerance of sudden salt stress and that homoeologous groups 4 and 6 play a role in the tolerance of gradually imposed salt stress. Homoeologous group 2 chromosomes reduced tolerance to either stress regime when their dose was increased. It should be noted that the data on homoeologous group 5 are inconclusive since only 1 of the 3 tetrasomics could be investigated.

The effects of the replacements of each of the three wheat homoeologous chromosomes by a specific *L. elongatum* chromosome may vary depending on the genotype of the replaced wheat chromosome. It seems reasonable to assume that the greater the significance of a wheat chromosome for salt stress tolerance, the smaller the enhancement of salt-stress tolerance due to its replacement with the *L. elongatum* homoeologue, and *vice versa*. Following this logic, the means of leaf elongation of DS lines in the A, B, and D genomes were averaged and statistically compared (Table 4). In plants stressed suddenly, DS lines involving the chromosomes of the B genome showed significantly greater salt stress tolerance than DS lines involving the chromosomes of the A or D genomes (Table 4). This suggests that the least significant contribution to the tolerance of sudden salt-stress is made by the B genome. The differences among the mean leaf elongation rates in the three genomes computed from DS lines stressed gradually were not statistically significant, although the A and D genome means were significantly higher than that of 'Chinese Spring' from day 3 to day 5 (Table 4).

Discussion

The observation that plants of *L. elongatum,* wheat, and their amphiploid showed greater rates of leaf elongation when exposed gradually to salt stress than plants that were stressed suddenly indicates that a gradual exposure to lower levels of salt stress facilitates an acclimation to higher levels of salt stress and implies that at least a component of salt-stress tolerance is induced in these plants. A similar observation was made in sorghum (Amzallag et al. 1990). These observations agree with several lines of molecular evidence indicating that *L. elongatum,* wheat, and likely most other grasses genetically acclimate to salt stress (Gulick and Dvorak 1987; Hurkman and Tanaka 1987; Ramagopal 1987; Skriver and Mundy 1990; Gulick and Dvorak 1992; Galvez et al. 1993).

Lophopyrum elongatum showed a much higher tolerance to either regime of salt stress than wheat, and its genome partially conferred this tolerance on wheat in the amphiploid. Cytogenetic analysis suggested that the tolerance of the two stress regimes may be based on common genetic and physiologic mechanisms, but, that superimposed on these common mechanisms are genetic and physiologic mechanisms that appear to be unique to each form of stress. When the data on DA and DTA lines are combined, the tolerance of sudden stress appears to be influenced by all *L. elongatum* chromosomes except for 4E; chromosomes 3E and 5E have the greatest influence. The tolerance of gradually imposed stress appears to be influenced by chromosomes 2E, 3E, 4E, 5E, and 7E, with chromosomes 3E, 4E, and 5E having the greatest influence. Thus, the tolerance of the two regimes have chromosomes 2E, 3E, 5E, and 7E in common. The physiological mechanisms influenced by chromosomes 1E and 6E seem to be more significant for plants stressed suddenly than for those stressed gradually, whereas the reverse appears to be true for the physiological mechanism influenced by chromosome 4E.

Several observations reinforce the idea that the tolerance of both regimes may have a common genetic base. Hyperploidy in DA line 2E had detrimental effects on the tolerance of both stress regimes. The euploidy for group 2 chromosomes, as in DS2E(2B), showed an opposite effect and resulted in an increased tolerance of both stress regimes. Neither arm of chromosome 3E enhanced tolerance of either stress regime. Both arms of 5E and 7E enhanced the tolerance of both stress regimes. In this case, however, DS5E(5B) only enhanced the tolerance of the sudden salt stress, suggesting that some genes on chromosomes in homoeologous group 5 may specifically affect sudden salt stress.

If the physiological mechanism of the tolerance of sudden and gradual salt stress has a large genetic component in common, why do plants tolerate the latter salt stress better than the former? The answer probably is in the genetic induction of the defense mechanism against salt stress. Accumulation of the mRNAs of the early-salt-stress gene systems becomes apparent 2 h and peaks about 6 h after the onset of salt stress (Gulick and Dvorak 1992; Galvez et al. 1993). Thus, if stress occurs suddenly, cells are not protected against the detrimental effects of high levels of salt stress for at least several hours.

The present data provide evidence that at least part of the genetic and physiologic mechanisms that are responsible for the superior tolerance of sudden and gradual salt stress in *L. elongatum* likely evolved by enhancement of mechanisms already operating in the common ancestor of *L. elongatum* and wheat. This is evidenced by the observation that, in several instances, tetrasomy of wheat chromosomes had similar effects on salt tolerance as the addition of homoeologous *L. elongatum* chromosomes. The increase in the dose of chromosomes 3A, 3B, and 3D had similar, albeit less strong, effects on the tolerance of suddenly imposed salt stress as the addition of chromosome 3E. Tetrasomics 3B and 3D were investigated here and tetrasomic 3A in another set of experiments (J. Dvorak, unpublished); tetrasomic 3A was not available for the present experiments. Addition of the complete or telocentric 1E and 7E chromosomes enhanced the tolerance of sudden salt stress. Increase in the dose of wheat chromosomes in both homoeologous groups showed similar effects; enhanced tolerance was found in tetrasomics 1A and 7A. The addition of chromosome 2E reduced tolerance of both stress regimes. Similar reductions of tolerance were observed when the dose of wheat homoeologues 2B and 2D was increased. Interestingly, tetrasomy for chromosome 2A did not have this effect, suggesting that the gene(s) responsible for this effect is absent from chromosome 2A. The effect of the addition of chromosome 4E on gradually imposed salt stress was paralleled by the effect of the increased dose of the only chromosome in the homoeologous group available for study, 4D.

The tolerance of salt stress conferred on wheat by *L. elongatum* chromosomes did not always agree when comparisons were made among DA, DTA, and DS lines involv-

ing the same *L. elongatum* chromosome. For example, DA6E showed no enhancement of the tolerance of sudden salt stress, but DTA for the long arm of this chromosome did. Disagreement between the expression of salt stress tolerance in DA and DS lines was particularly striking in homoeologous group 2, in which the DA line showed a lower tolerance than 'Chinese Spring' while DS2E(2B) resulted in an enhancement of tolerance. These conflicting findings are almost certainly manifestations of sensitivity to gene doses altered by aneuploidy. In agreement with our findings, wheat tetrasomics 2B and 2D and the DA line with added chromosome 2J of *Thinopyrum bessarabicum* also showed reduced salt tolerance (Forster et al. 1988). The dose sensitive locus is on chromosome arm 2EL; the arm location of the genes in wheat could not be determined. These findings emphasize that the use of both DA and DS lines generates a more relevant picture of the chromosomal control of complex traits, such as salt tolerance, than the use of either DS or DA lines alone (Dvorak et al. 1988).

Even then, some inconsistencies are to be expected. First of all, an alien chromosome may not fully compensate for the absence of a homoeologous wheat chromosome in a DS line, thus segmental aneuploidy may occur in DS lines even when the chromosome number is euploid. Additionally, wheat homoeologous chromosomes may differ either by the presence of different alleles, or structurally. Different DS lines involving the same *L. elongatum* chromosome may for these reasons differ in the expression of salt stress tolerance.

When wheat or *L. elongatum* are suddenly stressed with NaCl, they accumulate mRNAs of the *Esi* genes in the roots. The biphasic pattern of the steady-state mRNA accumulation and other lines of evidence suggest that these genes play the most critical role after sudden stress (Galvez et al. 1993). If this relationship exists, the *L. elongatum* chromosome arms, which are the locations of *Esi* genes in the *L. elongatum* genome, should confer tolerance of sudden salt stress on wheat. This is the case. Sudden stress tolerance was conferred on wheat by telosomes 1EL, 5ES, 5EL, 6EL, 7ES, and 7EL. Chromosome arms 1EL, 5EL, 6EL, and 7EL account for the location of 14 of the 16 *Esi* genes in the *L. elongatum* genome (Dubcovsky et al. 1994). The arms 1EL, 5EL, and 7EL also conferred tolerance of gradually imposed salt stress. Arm 5EL is the location of 5 of the 11 *Esi* loci identified, and it enhances the tolerance of both stress regimes more than any other *L. elongatum* chromosome arm.

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