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## Comparison of the genetic organization of the early salt-stress-response gene system in salt-tolerant *Lophopyrum elongatum* and salt-sensitive wheat

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**Abstract.** *Lophopyrum elongatum* is a facultative halophyte related to wheat. Eleven unique clones corresponding to genes showing enhanced mRNA accumulation in the early stages of salt stress were previously isolated from a *L. elongatum* salt-stressed-root cDNA library. The chromosomal distribution of genes complementary to these clones in several genomes of the tribe *Triticeae* and their copy number in the *L. elongatum* and wheat genomes are reported. Genes complementary to clones pESI4, pESI14, pESI15, pESI28, and pESI32 were found in homoeologous group 5, those complementary to pESI18 and pESI35 in homoeologous group 6, and those complementary to pESI47, pESI48, pESI3, and pESI2 in homoeologous groups 1, 3, 4, and 7, respectively. The genes are present in a single copy per genome in *L. elongatum* with the exception of those complementary to pESI2 and pESI18 which are present in at least two and five copies, respectively. Since similar copy numbers per genome were found in wheat (except for pESI2), the ability of *L. elongatum* to accumulate higher mRNA levels than wheat in response to salt shock appears to have evolved by changes in the regulation of these genes.

**Key words:** Salt stress – Osmotic stress – Gene number – Gene synteny – Wheat – *Lophopyrum* – Barley

### Introduction

*Lophopyrum elongatum* (Host) Á. Löve ( $2n = 2x = 14$ , genomes EE) is native to the Mediterranean region

where it grows in salt marshes and is very salt tolerant (McGuire and Dvořák 1981). *L. elongatum* was hybridized with bread wheat, *Triticum aestivum* L. ( $2n = 6x = 42$ , genome formula AABBDD), cultivar Chinese Spring, and a stable octoploid amphiploid,  $2n = 8x = 56$ , was produced (A. Mochizuki and B. C. Jenkins, unpublished). The amphiploid was shown to be more salt tolerant than the parental Chinese Spring wheat in both solution cultures (Dvořák and Ross 1986) and under field conditions (Omielan et al. 1991), showing that salt tolerance of *Lophopyrum* is expressed in a wheat genetic background.

Physiological studies in *L. elongatum* and wheat showed that tolerance of salt stress was higher if plants were exposed gradually to salt than if they were shocked (Dvořák et al. 1992). This suggested that growth under salt stress requires changes in gene expression (Gulick and Dvořák 1987). This was substantiated by isolation of partial cDNA clones of 11 different genes showing increased steady-state mRNA levels in response to salt stress. These clones were isolated from a *L. elongatum* cDNA library that was constructed from a mRNA population extracted from roots stressed with salt for 6 h (Gulick and Dvořák 1990). The mRNAs of these genes rapidly accumulate in *L. elongatum* roots in response to exposure to salt stress and reach peak levels within 6–12 h after the exposure to salt (Gulick and Dvořák 1992). Because of the enhanced expression of these genes shortly after an exposure to salt, they were named “early salt-induced” (*Esi*, Gulick and Dvořák 1992). The genes appear to be co-regulated (Gulick and Dvořák 1992), to respond primarily to osmotic shock, and be inducible by abscisic acid (ABA) (Galvez et al. 1993).

Sequencing of the clones (Gulick and Dvořák 1992) indicated that genes complementary to pESI18 and

pESI35 are distantly related to each other. The former clone is closely related to the *dhn3* clone isolated from desiccated barley seedlings by Chandler et al. (1988) and to clone *rab16* isolated by Mundy and Chua (1988) from ABA-treated rice. Both pESI18 and pESI35 are also related to clones *pcC6-19* and *pcC27-04* isolated from a water-stressed resurrection plant (Piatkowski et al. 1990). The remaining nine partial cDNA clones showed no homology to each other or any other reported DNA sequence (Gulick and Dvořák 1992). However, because the sequences are incomplete, homology in the unsequenced portions of the genes cannot be ruled out.

A prerequisite for investigation of the relationships between the tolerance to salt stress or osmotic shock imparted on wheat by individual *L. elongatum* chromosomes and the expression of the *Esi* genes in *L. elongatum* and wheat genomes is knowledge of the chromosomal distribution of the *Esi* genes. In this paper the chromosomal distribution of genes complementary to all 11 clones is reported for *L. elongatum*, barley and wheat.

When exposed to salt shock, the roots of *L. elongatum* accumulate greater amounts of *Esi* mRNAs than do those of wheat (Galvez et al. 1993). It is possible that the higher levels of mRNAs in *L. elongatum* compared to wheat reflect higher gene expression or higher mRNA stability, or both, in *L. elongatum* than in wheat. Alternatively, it is possible that the higher mRNA levels in *L. elongatum* reflect higher numbers of copies of these genes in the halophyte. To investigate this question, the number of copies of genes complementary to the 11 clones was determined in both species.

## Materials and methods

### Genetic stocks

The disomic substitution lines are designated DS and the ditelosomic substitution lines are designated DTS, followed by specifying the *Lophopyrum* chromosome or telosome present in the line. The replaced wheat chromosome is indicated in parenthesis. Disomic addition lines are designated DA and ditelosomic addition lines are designated DTA, followed by the added alien chromosome or telosome. Wheat ditelosomics are designated DT. Disomic substitution lines of *L. elongatum* chromosomes were produced by Dvořák (1980) and Dvořák and Chen (1984), except for DS3E(3A), DS3E(3B) and DS3E(3D), and DTS5EL(5A), DS5E(5B) and DS5E(5D), which were produced by Tuleen and Hart (1988). Ditelosomic addition lines 1ES, 2EL, 3ES, 4EL (= 4E $\alpha$ ), 5ES, 6ES, 7ES (= 7E $\beta$ ), and 7EL (= 7E $\alpha$ ) were produced by Dvořák (1979), and lines 1EL, 2ES, 3EL, 5EL, and 6EL by Hart and Tuleen (1983a). Chinese Spring DTs were produced by Sears and Sears (1979) and barley DAs by Islam et al. (1981). Individual barley chromosomes are designated according to their homoeology with wheat (Hart and Tuleen 1983b). Chromosomes originally known as 4A and 4B have been redesignated 4B and 4A<sup>a</sup>, respectively, as proposed by Dvořák et al. (1990). Sources of the accessions of the species of the tribe *Triticeae* used in the present study are listed in Table 1.

### DNA hybridization

Nuclear DNAs were isolated from leaves of single plants following the procedure of Dvořák et al. (1988). Restriction endonuclease-digested DNAs were electrophoretically fractionated in 1% agarose gel and transferred to Hybond N+ nylon membranes (Amersham) by capillary transfer. Prehybridization and hybridization were performed in a rotary hybridization chamber (National Labnet Company) at 65 °C in a solution containing 1% sodium dodecyl sulphate (SDS), 2.5 × SSPE buffer, 0.1% polyethylsulfonic acid, and 0.01% sodium pyrophosphate.

**Table 1.** Sources of the species of the tribe *Triticeae*

Species	Accession	Source	Origin
<i>Triticum urartu</i> Thum.	G3135	R. Johnson	Lebanon
<i>T. monococcum</i> L.	G2528	R. Johnson	Iran
<i>T. speltoides</i> (Tausch.) Gren.	TS02	M. Feldman	Israel
<i>T. sharonense</i> (syn. <i>Aegilops sharonensis</i> Eig.)	TH01	M. Feldman	Israel
<i>T. longissimum</i> (Sweinf. et Muschl.) Bowden	TL17	M. Feldman	Israel
<i>T. bicornis</i> Forssk	TB10	M. Feldman	Israel
<i>T. searsii</i> (syn. <i>Aegilops searsii</i> Feldman et Kislev)	TE27	M. Feldman	Israel
<i>T. muticum</i> (Bois.) Hackel	TK136-737	R. J. Metzger	Turkey
<i>T. caudatum</i> (L.) Godron et Gren.	Rub 74	E. R. Sears	Unknown
<i>T. comosum</i> (Sibth. et Smith) Richter	G659	G. Waines	Unknown
<i>T. uniaristatum</i> (Vis.) Richter	G3586	G. Waines	Unknown
<i>T. tauschii</i> (Coss.) Schmalh.	2075	Kyoto Univ.	Iran
<i>Heteranthelium piliferum</i> (Banks et Solander) Hochst	PI401352	D. Dewey	Iran
<i>Taeniatherum caput-medusae</i> (L.) Nevski	PI283270	D. Dewey	USSR
<i>Hordeum vulgare</i> L.	cv Betzes	A. K. R. M. Islam	Unknown
<i>Secale cereale</i> L.	cv Petkus	B. D. Fowler	Unknown
<i>Dasyphyron villosum</i> (L.) Candargy	88-2a	P. McGuire	Italy
<i>Agropyron cristatum</i> (L.) Gaertner	PI229574	R. Wang	Iran
<i>Lophopyron elongatum</i> (Host) Löve	e4	Unknown	Unknown
<i>Pseudoroegneria stipifolia</i> (Czern. ex Nevski) Löve	PI 313960	D. Dewey	USSR
<i>Thinopyrum bessarabicum</i> (Savul. et Rayss) Löve	D-3483	D. Dewey	USSR
<i>Psathyrostachys juncea</i> (Fisch.) Nevski	PI314668	D. Dewey	USSR

The immobilized DNAs were hybridized overnight with probes [ $\alpha$ - $^{32}$ P]-labeled by the random primer method (Amersham). To prepare probes, 11 ESI plasmids (Gulick and Dvořák 1990) were isolated from *Escherichia coli* (DH5 $\alpha$ ), digested with the endonucleases *Xba*I and *Sst*I, electrophoretically fractionated and the inserted cDNAs purified by electroelution. The membranes were washed in  $2 \times$  SSC and 0.5% SDS for 30 min,  $1 \times$  SSC and 0.5% SDS for 30 min, and  $0.5 \times$  SSC and 0.5% SDS for 30 min at 65 °C. Autoradiograms were exposed for 15 days at -70 °C with two intensifying screens.

#### Gene copy number determination

Ten-microgram samples of *L. elongatum* nuclear DNA were digested with four-base-pair recognizing endonucleases. Digested DNAs were fractionated by electrophoresis and transferred onto Hybond N+ along with amounts of cDNA insert equal to a single, two, five and ten gene copies per 10  $\mu$ g of genomic DNA. These copy number equivalents were calculated assuming the 2 C genome size of *L. elongatum* to be 12.3 pg (Bennett 1972). For electrophoresis, inserts were mixed with 5  $\mu$ g of sonicated salmon sperm DNA. The same cDNA inserts were used as probes. Band intensities in the autoradiograms were integrated using a laser scanning densitometer (Zeineh Model SL-TRFF). The number of copies of each *Esi* gene was estimated by comparison of the hybridization signals in genomic DNA with those in the gene-copy equivalents. The estimates (3–6 per clone) obtained for different restriction endonucleases were averaged. The number of copies in wheat were estimated by a quantitative comparison of the hybridization signals in wheat bands with *L. elongatum* bands within the same lanes in Southern blots of DNAs of disomic and ditelosomic addition and substitution lines. Estimates are means of measurements of 2–8 lanes.

#### Polymerase chain reaction (PCR)

Twenty nanograms of nuclear DNA from *L. elongatum*, *Hordeum vulgare*, *Secale cereale* (Table 1) and *T. aestivum*, cultivar Chinese Spring, were subjected to PCR for 45 cycles in a 100- $\mu$ l reaction volume containing two units of *Taq*I DNA polymerase (Promega),  $1 \times$  *Taq*I PCR Buffer (Promega), 0.2  $\mu$ M of each primer, and 200  $\mu$ M of each deoxyribonucleotide (Pharmacia). The liquid was overlaid with 50  $\mu$ l of mineral oil. The denaturation, annealing and primer extension steps were 94 °C for 1 min, 55 °C for 2 min, and 72 °C for 2 min, respectively. For pESI15 the annealing temperature was 45 °C. The primers were based on the unpublished sequences of the pESI clones (P. Gulick, personal communication). The sequences of the primers, the lengths of the PCR products and the distances to the poly-A tail are described in Table 2. Amplified products were blotted and probed with the electroeluted inserts to confirm their identity.

The PCR products, either intact or digested with a restriction endonuclease, were electrophoretically fractionated in 3.5% agarose gels.

## Results

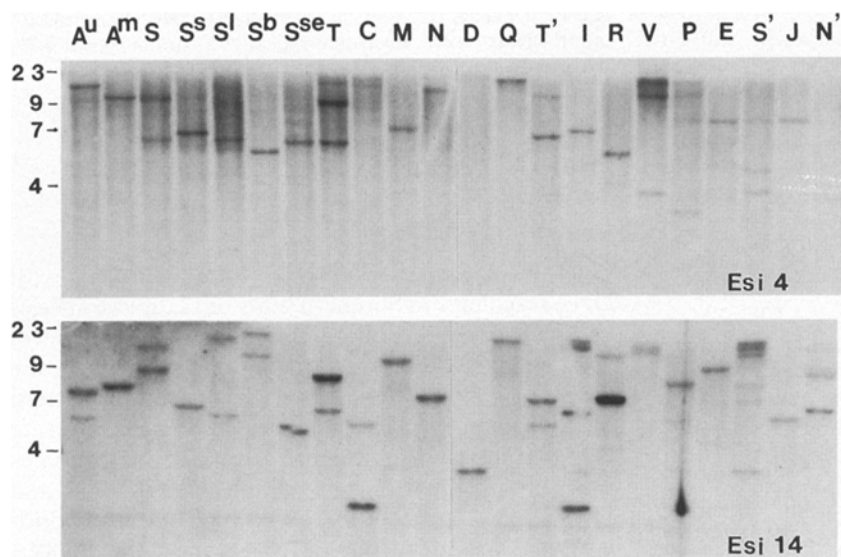
### Presence of the *Esi* genes in *Triticeae*

Each of the 11 partial cDNA clones hybridized with DNA fragments in Southern blots of 12 diploid species of *Triticum* and diploid species of 10 genera of *Triticeae*, indicating their ubiquitous presence in the tribe (Fig. 1). Each clone hybridized to a unique pattern of DNA fragments. This indicates that the 11 clones are cDNA transcripts of different genes and substantiates the results of the sequencing of the clones (Gulick and Dvořák 1992). Except for pESI18, the clones hybridized usually with one or two (or at most three) DNA fragments in the blots of DNAs of the majority of the diploid species (Fig. 1). The exceptions were pESI14, which hybridized with five fragments in barley DNA, six in *Pseudoroegneria* DNA, and four in *Psathyrostachys* DNA (Fig. 1), and pESI47, which hybridized with four fragments in *Triticum bicornis* DNA. This suggests that in most diploid species of the tribe these clones have a single genomic counterpart. Clone pESI18 hybridized with 4–10 fragments, indicating that it has several genomic homologues in each of the investigated genomes of the tribe.

It appears that the sequences of the genes have been evolving slowly. This is evident from the PCR amplification that was attempted for genes *Esi4*, *Esi14*, *Esi15*, and *Esi28* from genomic DNAs of *T. aestivum*, *L. elongatum*, *S. cereale*, and *H. vulgare* using sets of primers from the 3' ends of the *L. elongatum* genes and their 3' untranslated regions. Products were obtained with all four sets of primers. Hybridization of the products with the inserts electroeluted from the corresponding cDNA clones substantiated that they were indeed amplifications of the genomic copies of four different *Esi* genes. For each pair of primers, the PCR products were identical in length among the four spe-

**Table 2.** Primers, distance from the first nucleotide of the primer to the poly-A tail in *L. elongatum*, and PCR product lengths in the four species investigated

Primer	Primer sequence	Poly-A distance	Product length
pESI4	5' ACCTACGAGTGATTGGCTGA 3' 5'GGGAATTAAGAAGATAACGT	206 bp	206 bp
pESI14	5' CTGTAGGGGCTCCAACGTGC 3' 5'CAATTGTTCTTTAGCCACATC	342 bp	327 bp
pESI15	5' TTGATTCTTTTGTCTTACT 3' 5'GCAAAAATTTGGTTGATTTCT	137 bp	137 bp
pESI28	5' CTACCCTCGACATCCTCAAG 3' 5'CTCAATTATCAATCTCGAAG	199 bp	183 bp



**Fig. 1.** Southern-blot hybridization of the pESI4 and pESI14 inserts with *EcoRI*-digested DNAs of 12 diploid species of *Triticum* [*Triticum urartu* ( $A^u$ ), *T. monococcum* ( $A^m$ ), *T. speltoides* ( $S$ ), *T. sharonense* ( $S^s$ ), *T. longissimum* ( $S^l$ ), *T. bicorne* ( $S^b$ ), *T. searsii* ( $S^{se}$ ), *T. muticum* ( $T$ ), *T. caudatum* ( $C$ ), *T. comosum* ( $M$ ), *T. uniaristatum* ( $N$ ), *T. tauschii* ( $D$ )] and of other representative *Triticeae* species [*Heteranthelium piliferum* ( $Q$ ), *Taeniatherum caput-medusae* ( $T'$ ), *Hordeum vulgare* ( $I$ ), *Secale cereale* ( $R$ ), *Dasypyrum villosum* ( $V$ ), *Agropyron cristatum* ( $P$ ), *Lophopyron elongatum* ( $E$ ), *Pseudoroegneria stipifolia* ( $S'$ ), *Thinopyrum bessarabicum* ( $J$ ), *Psathyrostachys juncea* ( $N'$ )]. Molecular size markers from *HindIII*-digested bacteriophage  $\lambda$  are in kilobase pairs

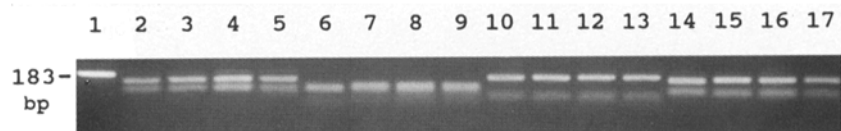
cies within the resolution of the agarose gels. The homology of the sequences between these four species in the amplified regions was scrutinized by digesting the PCR products with restriction endonucleases for which recognition sites were found in the *L. elongatum* sequences: pESI14 – *AclI*, and *TaqI*; pESI14 – *HinfI*, *MboI*, and *TaqI*; pESI15 – *AluI*, *DdeI*, *HinfI*, *RsaI*, *TaqI*; pESI28 – *AluI*, *BamHI*, *MboI*, *RsaI* and *TaqI*. No polymorphism was found among the species within the resolution of the agarose gels (Fig. 2).

#### Numbers of the Esi genes

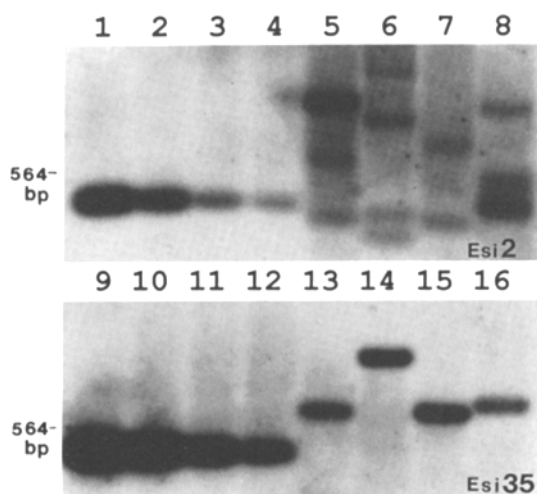
Hybridization of the inserts isolated from pESI clones with the *L. elongatum* nuclear DNA and equivalents of one, two, five and ten gene copies per 1C genome (Fig. 3) indicated that, except for pESI2 and pESI18, the clones had single genomic counterparts in the *L. elongatum* genome (Table 3). The mean hybridization intensity of pESI2 with *L. elongatum* DNA was

2.2 times greater than the intensity equivalent to single gene per 1C genome (Fig. 3). Three fragments, observed in *AluI* and *HaeIII* digests of *L. elongatum* nuclear DNA (Fig. 3), one of which showed twice as high signal level as the other two, probably originated by a cleavage of only one copy of the two genes, while four fragments observed in the *DdeI*-digested nuclear DNA (Fig. 3) probably originated by cleavage of *DdeI* sites in both copies.

The overall intensity of the hybridization signal of pESI18 corresponded to 4.7 copies per 1C genome. The signal in one band was equivalent to a single copy whereas its intensity in the other bands was less than a single copy per 1C genome. This indicates that there is a single homologous gene corresponding to pESI18 and four or more closely-related genes in the *L. elongatum* genome. The presence of more than one copy per genome of genes complementary to pESI2 and pESI18 is also indicated by the observation of several transcripts hybridizing with these clones in the Northern



**Fig. 2.** PCR amplifications of *Esi28* from *L. elongatum* (lanes 1, 2, 6, 10, 14), *T. aestivum* (lanes 3, 7, 11, 15), *S. cereale* (lanes 4, 8, 12, 16), and *H. vulgare* (lanes 5, 9, 13, 17). PCR amplifications of *Esi28* not digested (lane 1), digested with *BamHI* (lanes 2–5), *MboI* (lanes 6–9), *AluI* (10–13) and *RsaI* (lanes 14–17)



**Fig. 3.** Estimation of the copy number of *Esi2* (lanes 1–8) and *Esi35* (lanes 9–16) in *L. elongatum* by comparison of the signal of ten (lanes 1, 9), five (lanes 2, 10), two (lanes 3, 11) and one (lanes 4, 12) genomic copy equivalents, and *L. elongatum* genomic DNA digested with *AluI* (lanes 5, 15), *DdeI* (lane 6), *MboI* (lanes 7, 13), *HaeIII* (lanes 8, 14), and *TaqI* (lane 16)

blots (Galvez et al. 1993). The remaining pESI clones hybridized with only one transcript in Northern blots.

The intensities of wheat bands relative to the *L. elongatum* bands in the Southern blots of DNAs of disomic addition lines and disomic substitution lines (Table 3, Fig. 4) were quantitatively determined. Except for pESI2, the average intensity of the signal per genome in wheat did not differ significantly from the intensity of the signal in the *L. elongatum* bands, indicating that numbers of genes in the *Triticum* and *L. elongatum* genomes were similar (Table 3). For pESI2 the relative band intensities suggest that only one copy is present per wheat genome. Nevertheless, the possibility that at least one wheat genome has two copies cannot be ruled out because neither of the two wheat bands present in the *SstI*-digested DNAs of DS7E (7A), DS7E (7B) and DS7E (7D) completely disappeared (see below) and also because of two fragments were found in the profile of the *EcoRI*-digested DNA of *T. tauschii* (data not shown).

#### Chromosomal locations of *Esi* genes

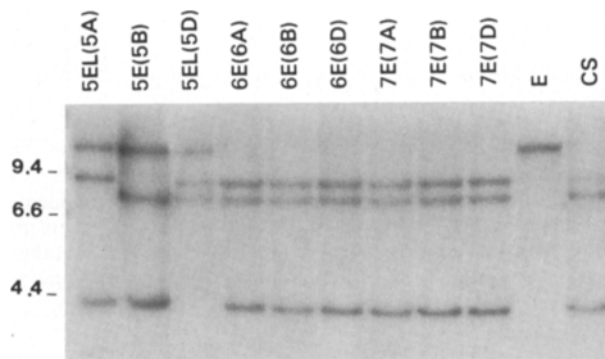
The chromosomal location in *L. elongatum* and wheat of genes complementary to the 11 clones was determined by hybridization of each clone with Southern blots of nuclear DNAs of the 21 *L. elongatum* disomic substitution lines. This strategy is illustrated in Fig. 4 using hybridization of pESI14 as an example. Hybridization of the clones with the Southern blots of nuclear DNAs of wheat ditelosomics and *L. elongatum* DTA

**Table 3.** Numbers of copies of *Esi* genes in *L. elongatum*, number of copies of *Esi* genes in *L. elongatum* relative to the average gene number per wheat genome, and maximum levels of mRNAs complementary to *Esi* genes in *L. elongatum* relative to wheat after shock with 250 mM of NaCl

Gene	No. of copies		mRNA levels relative to wheat <sup>a</sup>
	Absolute	Relative to wheat	
<i>Esi2</i>	2.2 ± 0.8 <sup>b</sup>	2.7 ± 0.4 <sup>b</sup>	5.3
<i>Esi3</i>	1.1 ± 0.3	1.2 ± 0.1	2.6
<i>Esi4</i>	1.0 ± 0.3	1.1 ± 0.1	1.2
<i>Esi14</i>	1.1 ± 0.2	0.9 ± 0.1	1.6
<i>Esi15</i>	0.5 ± 0.1	0.8 ± 0.1	2.0
<i>Esi18</i>	4.7 ± 0.5	1.0 ± 0.1	5.9
<i>Esi28</i>	1.1 ± 0.2	Not determined	2.1
<i>Esi32</i>	0.7 ± 0.3	1.0 ± 0.1	1.2
<i>Esi35</i>	1.0 ± 0.1	0.8 ± 0.3	2.5
<i>Esi47</i>	0.8 ± 0.2	1.0 ± 0.2	3.3
<i>Esi48</i>	1.1 ± 0.2	1.3 ± 0.1	3.5

<sup>a</sup> From Galvez et al. (1993)

<sup>b</sup> Standard error of the means



**Fig. 4.** Synteny mapping of *Esi14* using *L. elongatum* substitution lines and ditelosomic substitution lines. The replaced Chinese Spring chromosomes are indicated in parenthesis. Note the extra 12.7-kb *L. elongatum* *EcoRI* fragment in DTSS5EL(5A), DS5E(5B) and DTSS5EL(5D) and the absence of the wheat 7.6-, 9.0-, and 3.9-kb fragments, respectively, in the same DNAs. Only DNAs of chromosome substitution lines of homoeologous groups 5, 6 and 7 are shown. Those of homoeologous groups 1, 2, 3 and 4 (data not shown) gave a pattern identical to that of Chinese Spring (CS) and those of the substitution lines of homoeologous groups 6 and 7. Molecular size markers from *HindIII*-digested bacteriophage  $\lambda$  are in kb

lines was employed to place the genes into chromosome arms. A similar strategy was used for placing the genes into synteny groups in barley.

The results of the mapping of the restriction fragments are summarized in Table 4. The short lengths of several clones, notably pESI15 and pESI32, which

**Table 4.** Chromosomal location of the *Esi* loci based on the location of restriction fragments in *L. elongatum* – wheat disomic and ditelosomic addition lines and disomic substitution lines, and in barley-wheat disomic and ditelosomic addition lines

<i>Esi</i>	<i>L. elongatum</i> kb	Chrom.	Arm		wheat kb	Chrom.	Arm		Barley kb	Chrom.	Arm	
			S	L			S	L			S	L
2	3.1 + 6.5(S)	7E	–	+	4.1(S) 5.4(X) 6.8(X) 2.0(D)	7A, 7D 7A 7B 7B	N n.d. – n.d.	R + n.d. n.d.	1.7(S)	7H	n.d.	n.d.
3	1.7(S)	4E	n.d.	+	2.8(S) 1.9(S)	4B 4D	– –	n.d. n.d.	9.7(S)	4H	–	+
4	1.7(S)	5E	–	+	5.2(S) 6.1(S)	5A 5B	n.d. n.d.	+ +	n.d.	n.d.	n.d.	n.d.
14	12.7(E)	5E	–	+	7.6(E) 9.0(E) 3.9(E)	5A 5B 5D	n.d. n.d. n.d.	+ + +	n.d.	n.d.	n.d.	n.d.
15	7.2(S)	5E	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18	7.4(X)	6E	–	+	3.6(X) 5.0(X) 1.6, 3.4, 5.8(X)	6A 6B 6D	– – –	– + +	8.9(S)	6H	–	+
28	8.5(D)	5E	n.d.	+	6.1(D) 9.7(D)	5B 5D	n.d. n.d.	+ +	4.8(S)	5H	n.d.	n.d.
32	10.5(B)	5E	–	+	1.9(B) 5.6(B)	5A 5B	n.d. n.d.	+ +	n.d.	n.d.	n.d.	n.d.
35	13.8(S)	6E	–	+	9.9(S) 4.2(S) 5.9(S)	6A 6B 6D	– – –	+ + +	9.1(S)	6H	–	+
47	5.4(X)	1E	–	+	9.9(X) 15.7(X) 3.8(X)	1A 1B 1D	– – –	+ + +	n.d.	n.d.	n.d.	n.d.
48	1.0(BN)	3E	–	+	1.1(BN) 1.2(BN) 1.4(BN)	3A 3B 3D	n.d. n.d. n.d.	+ + +	n.d.	n.d.	n.d.	n.d.

Restriction fragments generated by digestion of genomic DNAs with *Bgl*III, *Bst*NI, *Dra*I, *Eco*RI, *Sst*I, and *Xba*I, are indicated by B, BN, D, E, S, and X in parenthesis, respectively. Restriction fragments mapped with additions and substitutions of complete chromosomes are indicated in the "Chrom." column. Restriction fragments mapped with telosomes are indicated by + (present in the arm) and – (absent in the arm) in the "Arm" column. n.d. indicates that the presence or absence of a fragment on a chromosome or chromosome arm was not determined. R and N indicate a reduced and normal copy number of a fragment, respectively

were only 125 and 152 bp long, respectively, made the Southern hybridization arduous and resulted in failure to associate a gene with a chromosome in several cases. A gene(s) complementary to pESI2 was mapped in the long arms of chromosomes 7E, 7A, 7B and 7D (Table 4). Nevertheless, no missing fragments were found in the *Sst*I, *Xba*I, or *Dra*I digestions of DS7E(7D) or DT7DS, and the 7DL location of the gene based only on lower intensity of the *Sst*I band. Fragments hybridizing with pESI3 were found in the long arms of chromosomes 4E, 4B and 4D (Table 4). No fragment hybridizing with pESI3 was deleted as a consequence of the absence of wheat chromosome 4A<sup>a</sup>, and there is, consequently, no evidence that this gene is in this wheat chromosome. *Esi*15 was mapped only in the long arm of chromosome 5E (Table 4). The short length of pESI15 resulted in failure to obtain a signal in Southern blots except for *Sst*I digests. In this hybridization a single 1.7-kb wheat fragment was observed. Since the fragment was present in all disomic substitu-

tion lines it could not be assigned to a wheat chromosome. Genes complementary to pESI18 are in the long arms of chromosomes 6E, 6A, 6B and 6D (Table 4). An anomaly was observed in ditelosomic 6AL. A 3.6-kb Chinese Spring band absent from the DNA of DS6E(6A) was also absent in both Chinese Spring ditelosomics for chromosome 6A. A novel 9.6-kb band, not present in Chinese Spring, was observed in DT6AL. Polymorphisms for this ditelosomic line were also reported by Anderson et al. (1992). *Esi*32 was not unequivocally assigned into a synteny group in barley, but the presence of a faint 8.3-kb Betzes band in DA5H and DTA5HL suggested that a gene complementary to pESI32 is also present in the long arm of chromosome 5H in barley.

## Discussion

Genes complementary to clones pESI4, pESI14, pESI15, pESI28, and pESI32 were found in ho-

moeologous group 5, those complementary to pESI18 and pESI35 in homoeologous group 6, and the other four, complementary to pESI47, pESI48, pESI3, and pESI2, in homoeologous groups 1, 3, 4, and 7, respectively. Nine of the genes complementary to the 11 cDNA clones appeared to be present only once in the *L. elongatum* genome while those complementary to pESI2 and pESI18 appeared to be present in multiple copies. A minimum estimate of the total number of *Esi* genes in the *L. elongatum* genome was 16. Although the genes were on six of the seven *L. elongatum* chromosomes they were significantly clustered; 11 of them were in chromosomes 5E and 6E.

The genes in chromosome 6E were related to each other. The DNA sequences of pESI18 and pESI35 showed 70% homology (P. Gulick, personal communication). The truncated sequence of 39 amino acids of the C terminal region of pESI18 showed 80% homology to barley dehydrin *dhn3* (Gulik and Dvořák 1992). Barley dehydrin genes *dhn3* and *dhn4* are in barley chromosome 6 (Close and Chandler 1990; Heun et al. 1991) but the linkage relationships of these genes to genes homologous with pESI18 and pESI35 is not known. In addition to the genes in 6H, Close and Chandler (1990) reported two additional genes (*dhn1* and *dhn2*) in chromosome 5H. The homology and linkage relationship between these two genes and the five *Esi* genes mapped to synteny group 5 here is not known and will require sequencing of the full-length clones and linkage analysis. The cDNA clones of the five genes in the 5EL synteny group showed no homology to each other. However, these are short partial clones and it is possible that some homology may exist in the unsequenced portions of the genes. In spite of the limited sequence information about these genes, the data on PCR amplification and Southern hybridization of the clones with representative species of *Triticeae* leave little doubt that the origin of the genes in chromosome 5 preceded the divergence of the tribe. The same must be true for the divergence of *Esi35* from the *Esi18* genes in the 6EL synteny group. The genes complementary to each of the remaining four clones were found to be in homoeologous chromosomes in *Lophopyrum*, *Triticum*, and *Hordeum* and their origin must also precede the divergence of the tribe. Obviously, the early salt stress response is controlled by an ancient gene system that evolved before the origin of the tribe *Triticeae*.

*L. elongatum* shows generally higher mRNA levels of the *Esi* genes than wheat when exposed to the same level of salt shock (Galvez et al. 1993). Since the numbers of copies per genome of all genes, except for *Esi2*, were the same in *L. elongatum* and wheat, higher mRNA accumulation in *L. elongatum* (Table 3) did not evolve by copy-number increases but by either a higher

transcription rate, a higher stability of the steady-state mRNA, or both.

An exception are the genes complementary to pESI2. The hybridization with genomic equivalents and the pattern of restriction fragments indicated that there are at least two copies complementary to pESI2 in the *L. elongatum* genome but on the average only one wheat genome. It is clear that the two *L. elongatum* copies have diverged from each other since they differ in some restriction sites (Fig. 3). Galvez et al. (1993) reported three transcripts hybridizing with pESI2 in KCL-stressed *L. elongatum*.

The finding that the *Esi* genes are ubiquitous in *Triticeae* and that their copy numbers do not greatly vary among the species of the tribe, irrespective of whether the species are salt-tolerant or not, suggests that the evolution of halophytism in *L. elongatum* was not associated with the evolution of new genes but most likely was involved with changes in the regulation of an ancient repertoire of genes already fully evolved in the ancestral glycophyte.

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