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Linkage relationships among stress-induced genes in wheat

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Abstract Linkage relationships among genes responding to water-deficit, salt stress, and heat shock were investigated in diploid wheat, *Triticum monococcum* L. The position of these gene loci relative to closely linked markers and the centromeres is reported. It is proposed to continue to use the present *T. monococcum* mapping population and the genetic maps based thereon as a framework for future determination of relationships among other genes related to environmental stress in the tribe Triticeae.

Key words Salt stress · Water deficient · Heat shock · Mapping · Triticeae · RFLP · Linkage map

Introduction

The exposure of plants to environmental stresses alters the accumulation of mRNAs of stress-induced genes. The relationships among stress-induced genes have been increasingly more difficult to grasp as the number of such genes continues to increase. Moreover, these genes have been isolated from different species, via expression in different tissues and in response to different stress regimes.

In the tribe Triticeae, clones of genes which show steady-state mRNA accumulation in response to water deficit, salt stress and heat stress have been isolated. Water-deficit-induced genes have been isolated as cDNAs from desiccating barley seedlings (dehydrin clones, Close et al. 1989; Close and Chandler 1990), desiccating wheat roots (clone pTtu1937=pWSP16, King et al. 1992), and desiccating or imbibed wheat or barley seeds (clone p1015 of early-methionine-labelled polypeptide, Cuming 1984; Williamson et al. 1985; pB19 clones, Espelund et al. 1992; and clones of dormins, Morris et al. 1991). Messenger RNA of wheat-germ agglutinin (lectin) was shown to accumulate in wheat roots in response to desiccation (Cammue et al. 1989). Virtually all these genes have also been shown to be abscisic acid (ABA)-responsive (Cammue et al. 1989; Morris et al. 1990, Morris et al. 1991; Bostock and Quatrano 1992; Espelund et al. 1992). Eleven different cDNA clones have been isolated from salt-stressed roots of *Lophopyrum elongatum* (Host) A. Love (Gulick and Dvořák 1990). These genes appear to be coregulated and show rapid steady-state mRNA accumulation in the first 12 h of salt stress; hence, this group of genes was designated as early-salt-induced (*Esi*) (Gulick and Dvořák 1990, 1992). Each of these genes has also been shown to be ABA-responsive (Galvez et al. 1993), which potentially associates them with the water-deficit-induced genes. Germine (which is oxalate oxidase, Lane et al. 1993), has been shown to accumulate in the roots of salt-stressed barley (Hurkman et al. 1994), and the 70-kDa subunit (A-subunit) of the vacuolar ATPase (V-ATPase) has been shown to accumulate in salt-stressed and ABA-treated tobacco cells (Narasinham et al. 1991). A clone of the A-subunit of V-ATPase (F. M. DuPont, unpublished) and two clones of the B-subunit of V-ATPase (Berkelman et al. 1994) have been isolated from barley. Finally, several heat-shock-induced genes of the low-molecular-weight (LMW) class, ranging in size from 16 to 26 kDa, have been isolated from wheat (Weng et al. 1991a, b, 1993).

Information about the distribution of stress-induced genes in Triticeae genomes varies. Precise linkage information exists in barley (Kleinhofs et al. 1993; Kleinhofs et al. in Matthews and Anderson 1994) for dehydrin loci *Dhn2* (barley chromosome arm 5HL), *Dhn3* and *Dhn5* (6HL), and *Dhn6* (4HS); dormin loci *Dor2* (1HL), *Dor4* (3HL), and *Dor5* (5HS); and *VAtp-B1* (7HL). Linkage information also exists for the early-salt-induced gene 47 (*XEsi47*), early-methionine-labelled polypeptide locus (*XEm*), and wheat germ agglutinin (*XLec*) in *T. monococcum* chromosome arm $1A^mL$ (Dubcovsky et al. 1995), for the latter two loci in rye chromosome arm 1RL (Wang et al. 1991), and for the oxalate oxidase locus (*XGer*) on wheat chromosome arms 4AL, 4BS, and 4DS (Lagudah et

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al. 1991; Devos et al. 1995). Only the chromosome arm location is known for most of the *Esi* genes (Dubcovsky et al. 1994) and genes encoding the LMW heat-shock proteins (Porter et al. 1989). Finally, no genetic information is available for the wheat water-deficiency-induced protein pTtu1937(WSP16) and the V-ATPase subunits A and B2.

Chromosome arm mapping of dehydrin and *Esi* genes has indicated that large number of these genes are clustered in chromosomes 5 and 6; genes complementary to 2 dehydrin clones (pTz19R-dhn1 and pTz19R-dhn2) and 5 ESI clones (pESI4, pESI14, pESI15, pESI28, and pESI32) were assigned to chromosomes in homoeologous group 5, and those complementary to 3 dehydrin clones (pTz19R-dhn3, 4, and 5) and 2 ESI clones (pESI18 and pESI 35) were assigned to chromosomes in homoeologous group 6 (Close and Chandler 1990; Kleinhofs et al. in Matthews and Anderson 1994; Dubcovsky et al. 1994). Since salt stress and water-deficit stress are physiologically related, it is possible that *Esi* and *Dhn* genes are members of the same multigene loci present in chromosomes 5 and 6. Although the sequences of the 11 ESI clones differ from each other (Gulick and Dvořák 1990), the possibility of some homology can not be ruled out because most of the clones are only truncated sequences of the 3' regions of the genes (except for pESI3 and pESI35). The complete sequence of pESI3, and from it the inferred amino acid sequence, showed that *Esi3* encodes a small hydrophobic protein (Gulick et al. 1994). Clone pESI3 shows a high similarity to a barley gene induced by low temperature (Goddard et al. 1993). The nucleotide sequence of pESI35 suggests that *Esi35* encodes a hydrophilic protein distantly related to the dehydrins (Gulick and An 1993).

We determined the linkage relationships of stress-induced genes, with the main emphasis on the *Esi* genes, in the genome of diploid wheat, *Triticum monococcum* L. (2n=2x=14, genomes A^mA^m). The positions of the gene loci relative to closely linked molecular markers in the *T. monococcum* map were also determined. *T. monococcum* is a diploid wheat closely related to polyploid durum and bread wheats. It was chosen for this work because it is more polymorphic than the polyploid wheats, which facilitated efficient molecular mapping.

Materials and methods

Two F_2 populations of *T. monococcum* were used for mapping. The first one included 75 F_3 families from a cross between two wild *T. monococcum* ssp. *aegilopoides* accessions from Turkey (G1777) and Iran (G2528). This population was used only to map the clones in chromosome 1. Mapped loci were assigned to chromosome arms using ditelosomic substitution lines of the short and long arms of chromosome $1A^m$ G1777 in *T. aestivum* cv. 'Chinese Spring', abbreviated as DTS1A^{m}L(1A) and DTS1A^{m}S(1A), respectively. The second, more polymorphic, mapping population included 76 F_2 individuals from a cross between cultivated *T. monococcum* spp. *monococcum* DV92 from Monte Negro and *T. monococcum* ssp. *aegilopoides* G3116 from Lebanon. In this population, the approximate positions of the centromeres were inferred by employing clones of loci that have been placed into opposite arms in bread wheat and barley linkage maps by telosomic mapping (Anderson et al. 1992; Kleinhofs et al. 1993; Dubcovsky et al. 1994).

Table 1 Gene loci detected with the environmental-stress-related clones, cross hybridization with DNA fragments from these loci with other clones, and the general class of proteins encoded at the gene loci

Locus	Clone	Other clones hybridizing with the same locus	Class
XEsi2, 3, 4, 14, 28, 32, 47, 48	pESI2, 3, 4, 14, 28, 32, 47, 48[a]	None	Unknown
XEsi35	pESI35[a]	None	Distantly related to LEA group 2
XDhn2.1	pTZ19R-dhn2[b]	pTZ19R-dhn1[b], 2; pTtu1937(WSBP16)[c]	LEA group 2
XDhn2.2	pTZI19R-dhn2	pTZI19R-dhn1, 2, 3, 4, 5, 6[b]; pTtu1937(WSP16); pESI18-1[d]	LEA group 2
XDhn3	pESI18-1	pTZ19R-dhn 2, 3, 4, 5; pTtu1937(WSP16)	LEA group 2
XDhn6	pTZ19R-dhn6	pTZ19R-dhn2	LEA group 2
XEm	p1015[e]	pMA1959[f], pB19.1[g]	LEA group 1
XDor4	pMA1949[f]	None	LEA group 3
XDor5	pMA1951[f]	None	Unknown
XLec	pNVR20[h]	None	Wheat-germ agglutinin
XGer-2A^m	pWJHGermin[i]	None	Oxalate oxidase
XGer-4A^m	pWJHGermin[i]	None	Oxalate oxidase
XVAtpA	pHTA[j]	None	V-ATPase subunit A
XVAtp-B1	pHTB1[k]	None	V-ATPase subunit B
XVAtp-B2	pHTB2[k]	None	V-ATPase subunit B
Xttu1934(Hsp16.9b)-3A^m	pTtu1934(Hsp16.9b)[l]	None	L-M-W heat-shock proteins
Xttu1934(Hsp16.9b)-5A^m	pTtu1934(Hsp16.9b)	None	L-M-W heat shock proteins
Xttu1935(Hsp17.3)	pTtu1935(Hsp17.3)[m]	None	L-M-W heat-chock proteins
Xttu1936(Hsp26.6a)	pTtu1936(Hsp26.6a)[n]	None	Chloroplast heat-shock proteins

[a] Gulick and Dvořák (1990), [b] Close and Chandler (1990), [c] King et al. (1992), [d] full length clone of pESI18 (P. E. Gulick, unpublished), [e] Williamson et al. (1985), [f] Morris et al. (1991), [g] Espelund et al. (1992), [h] Reikhel and Wilkins (1987), [i] Hurkman (1994), [j] F. M. DuPont (unpublished), [k] Berkelman et al. (1994), [l] Weng et al. (1993), [m] Weng et al. (1991b), [n] Weng et al. (1991a)

Twenty-eight clones were employed in mapping of stress-induced genes (Table 1, Fig. 1). Their positions relative to 38 other markers (Table 1, Fig. 1), which provide anchor points within the molecular marker maps of barley, wheat, and *T. monococcum*, were determined.

Nuclear DNAs were isolated from leaves of single F_2 plants or from leaves of 10–20 pooled F_3 plants following the procedure of Dvořák et al. (1988). Southern hybridization was performed as described earlier (Dubcovsky et al. 1994). Maps were constructed using the Kosambi function (Kosambi 1943) and the computer program Mapmaker/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992).

Results and discussion

Chromosome 1

Genes complementary to the clones pNVR20, pESI47, p1015, pMA1959, and pB19.1 are all in the long arm of chromosome $1A^m$ since these clones hybridized with DNA fragments that were present in the hybridization profiles of DTS$1A^m$L but not in those of DTS$1A^m$S. Clones p1015, pB19.1, and pMA1959 are independent clones from locus *XEm* since they hybridized with the same set of restriction fragments and mapped to the same locus (Table 1, Fig. 1). Locus *XEsi47* is completely linked to the centromere and, across the centromere, to *Xcdo658* in the short arm of chromosome $1A^m$.

Chromosome 2

Barley clone pWJHGermin (Hurkman et al. 1994) is homologous to wheat germin (oxalate oxidase) clone gf-2.8 (Lane et al. 1992; 1993). The barley clone hybridized with from four to eight restriction fragments, depending on the restriction enzyme used. One pair of polymorphic fragments was mapped to the centromeric region of chromosome $2A^m$ (the arm location unknown) (Fig. 1). Another locus was mapped on chromosome $4A^m$.

Chromosome 3

Genes complementary to the heat-shock clones pTtu1934(Hsp16.9b) and pTtu1935(Hsp17.3), the early-salt-induced clone pESI48, and the dormin clone pMA1949 were mapped in this linkage group (Fig. 1). The position of *XEsi48* agrees with the previous work that placed the gene in the long arms of wheat chromosomes 3A, 3B, 3D, and *Lophopyrum elongatum* chromosome 3E (Dubcovsky et al. 1994). The locus is proximal to the locus *Xmwg571* and is completely linked to locus *Xabg377*, both of which have been previously mapped on chromosome arm 3HL (Kleinhofs et al. 1993, 1994 in Matthews and Anderson 1994). Locus *XDor4*, which is proximal to ESI48, was detected with the dormin clone pMA1949 and has also been previously mapped on chromosome arm 3HL (Kleinhofs et al. 1993).

Heat-shock protein locus *Xttu1935(Hsp17.3)* was assigned to the short arm of chromosome 3E of *L. elongatum* (data not shown) and 3D of bread wheat (M. D. Gale personal communication). This locus is completely linked to *Xpsr903*. Locus *Xttu1935(Hsp17.3)* appears to be in the centromeric region of the short arm of $3A^m$ because *Xpsr903* loci are on wheat chromosome arms 3AS, 3BS, and 3DS (Devos et al. 1993). The heat shock protein locus *Xttu1934(Hsp16.9b)* maps on the short arm of chromosome $3A^m$ and is completely linked to *Xabc171* (Fig. 1). This heat shock clone hybridized with a duplicate locus on chromosome arm $5A^m$L.

Porter et al. (1989) employed two-dimensional (2-D) isoelectric focusing (IEF)-SDS polyacrylamide gel electrophoresis (SDS-PAGE) for chromosome arm mapping of LMW heat-shock proteins in wheat ditelosomic stocks. Proteins of approximate molecular weights of 17–18 kDa were found to be encoded by chromosome arms 3AL, 3BS, and 3DS. In the B and D genomes, the position of these heat-shock protein loci agreed with the placements of the *Xttu1934(Hsp16.9b)* and *Xttu1935(Hsp17.3)* loci on $3A^m$S but disagreed with the inferred placements of a locus on the 3AL arm. If colinearity of wheat chromosome 3A and *T. monococcum* chromosome $3A^m$ is assumed, both heat-shock protein loci on bread wheat chromosome 3A should be in the short arm. Porter et al. (1989) stated that spot no. 12, which appeared to be associated with 3AL arm, was variable. It is therefore possible that the variation between DT3AS and DT3AL was caused by some factor, possibly expression of a regulatory gene (Porter et al. 1989), other than the location of a structural gene for spot no. 12 on arm 3AL.

The two heat-shock protein loci in the short arm of chromosome 3 encode different proteins: *Xttu1934(Hsp16.9b)* and *Xttu1935(Hsp17.3)* encode class I and class VI heat shock-proteins, respectively (Weng et al. 1991b, 1993).

Chromosome 4

Genes complementary to clones pTZ19R-dhn6, pESI3, and pTtu1936(HSP26.6a) are tightly linked in the centromeric region. Since probes pESI3 and pTt1936(HSP26.6a) hybridized with different DNA fragments, *XEsi3* and *Xttu1936(HSP26.6a)* must be different loci. A locus detected with clone pTZ19R-dhn6 (barley chromosome arm 4HS; Kleinhofs et al. 1993) was only 4.1 cM from locus *XEsi3*, which was previously found to be on arms 4EL, 4BL, and 4DL (Dubcovsky et al. 1994). Loci *XEsi3* and *Xttu1936(HSP26.6a)* were 0.7 cM distal to *Xabg484*, which is on 4HL (Kleinhofs et al. 1993), suggesting that *Xttu1936(HSP26.6a)* is also on the long arm of chromosome $4A^m$. Dehydrin probe pTZ19R-dhn6, which detects locus *XDhn6*, cross-hybridized with DNA fragments from locus *XDhn2.2* on chromosome $5A^m$. A *XGer-4A*^{m} locus was mapped on the short arm of chromosome $4A^m$. The position of this locus is similar to that previously reported by Lagudah et al. (Lagudah et al. 1991) for *T. tauschii* and Devos et al. (Devos et al. 1995) for wheat chromosomes 4B and 4D. The duplicate *XGer-2A*^{m} locus has not been reported.

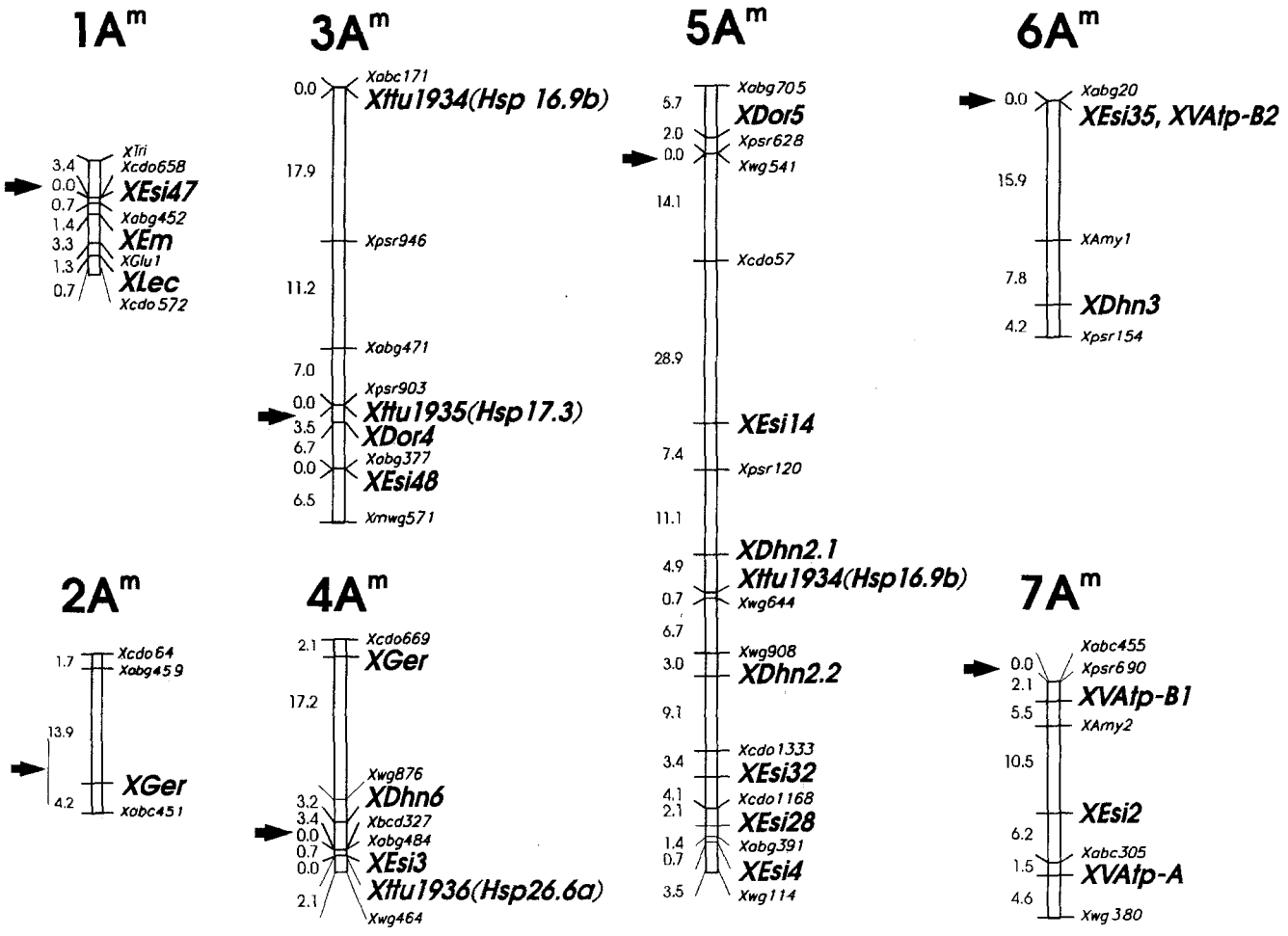

Fig. 1 Location of loci hybridizing with clones of environmental-stress-related genes (shown in *bold*) on the chromosomes of *Triticum monococcum*. Markers linked to each locus are also shown. The linked markers were detected with the following probes: *abc* and *abg* were respectively cDNA and genomic clones of unknown function from barley (Kleinhofs et al. 1993); *bcd* and *cdo* were cDNA clones of unknown function from barley and oat, respectively (Anderson et al. 1992); *wg* were genomic clones from wheat (Anderson et al. 1992); *mwg* was a genomic clone from barley (Graner et al. 1991); *psr* were cDNA or genomic clones from wheat (Devos et al. 1992; and M. D. Gale unpublished); *Chs* was a chalcone synthase clone (Rohde et al. 1991); the *Glu1* probe was pDY10A/KS$^-$ (Anderson et al. 1989); the *Amy1* probe was pHV19 (Khursheed and Rogers 1988). The approximate positions of the centromers are indicated by *arrows*

The location of heat-shock protein locus *Xttu1936(HSP26.6a)*, which encodes a 26- to 27-kDa chloroplast protein, on the long arm of chromosome 4A^m agrees with the chromosome arm mapping of genes encoding proteins of this molecular weight by 2-D IEF-SDS PAGE on 4BL and 4DL (Porter et al. 1989). However, there must be additional heat-shock protein genes on the long arm of chromosome 4 since Porter et al. (1989) could associate two 18-kDa proteins with arm 4DL. Genes encoding these proteins have presumably not been cloned, and it is not clear whether they are at the *Xttu1936(HSP26.6a)* locus or elsewhere on the chromosome.

Chromosome 5

The long arm of this chromosome has the highest concentration of stress-induced genes (Fig. 1). Of the 5 *Esi* genes previously placed on the long arm by chromosome arm mapping (Dubcovsky et al. 1994), 4 were mapped in a 54.6-cM region of the long arm. Mapping of the 5th ESI clone, pESI15, failed because of its small size (125 bp). Since the most distal locus in this region, *XEsi14*, was assigned to the long arm of chromosomes 5E, 5A, and 5B (Dubcovsky et al. 1994), the 54.6-cM region must be proximal to the breakpoint of the 4A^m/5A^m translocation that exists in *T. monococcum* (Devos et al. 1995). Locus *Xwg114*, previously mapped on chromosome arm 4HL (Kleinhofs et al. 1993), is distal to that breakpoint; the breakpoint resides between *XEsi4* and *Xwg114*. Each of the 4 ESI clones were from a different locus. All 4 *XEsi* loci differed from the 2 *XDhn* loci that exist in the region (Fig. 1).

The restriction fragments at the *XDhn2.1* locus hybridized with clones pTZ19R-dhn1, pTZ19R-dhn2 and, at a lower intensity, with pTtu1937(WSP16) (Table 1). The fragments assigned to the second locus, *XDhn2.2*, produced an intense signal with clone pTtu1937(WSP16), an intermediate one with pTZ19R-dhn3 and 4, and a weak one with clones pTZ19R-dhn1, 2, 5, and 6 and pESI18-1 (Ta-

ble 1). Clones pTZ19R-dhn2, 3, 4, and 5, pTtu1937 (Wsp16), and pESI18-1 also hybridized with DNA fragments that mapped on chromosome 6 (Table 1). The *XDhn2* locus was mapped on barley chromosome 5H in the Tr306×'Harrington' mapping population (Kleinhofs et al. in Matthews and Anderson 1994). The relationship of this locus to *Xwg644* and other markers indicated that the locus mapped in barley corresponds to *T. monococcum* locus *XDhn2.1*.

Dormin locus *XDor5* detected with clone pMA1951 was mapped near the centromere on the short arm of chromosome $5A^m$, which agrees with a similar position in barley chromosome arm 5HS (Kleinhofs et al. 1993).

The duplicate heat-shock locus *Xttu1934(Hsp16.9b)-5A* may not be expressed since Porter et al. (1989) did not report any spot in 2-D IEF SDS-PAGE to be associated with any wheat chromosome of homoeologous group 5.

Chromosome 6

Locus *XEsi35* mapped 15.9 cM proximal to *XAmy1* and was completely linked to *Xabg20*. Since *Xabg20* is on chromosome arm 6HS in barley (Kleinhofs et al. 1993) and *XEsi35* is on 6HL, 6EL, 6AL, 6BL, and 6DL (Dubcovsky et al. 1994), *Xabg20* and *XEsi35* are likely on the opposite sides of the centromere. Locus *XVAtp-B2*, which is complementary to the barley cDNA clone pHTB2 of the B subunit of V-ATPase, was completely linked to *XEsi35*. The fact that the pHTB2 and ESI35 probes hybridized with different restriction fragments showed that *XVAtp-B2* and *XEsi35* are different loci.

Clones pTZ19R-dhn3 and 4 and pTtu1937(WSP16) hybridized with the same set of restriction fragments. Clone pESI18-1 hybridized with a subset of the fragments detected with the previous clones. In *Dra*I-digested DNA, pESI18-1 hybridized with 13 fragments that also hybridized with the previous clones and with 1 fragment that hybridized only with the ESI18-1 probe. ESI18-1 did not hybridize with 2 fragments that hybridized with pTZ19R-dhn3 and 4 and pTtu1937(WSP16). The *Dra*I fragments common to these 4 clones cosegregated and mapped between loci *Xpsr154* and *XAmy1*, previously mapped on 6HL (Kleinhofs et al. 1993). The linkages of the *T. monococcum* locus detected with pESI18-1, pTZ19R-dhn3, pTtu1937(WSP16) and other dehydrin probes to the flanking markers showed that this locus corresponds to the *Dhn3* locus that already has been mapped in the barley mapping population TR306×Harrington (Kleinhofs et al. in Matthews and Anderson 1994); therefore, the *T. monococcum* locus will be designated *XDhn3*. Another dehydrin locus, *Dhn5*, has been mapped completely linked to *Dhn3* in barley chromosome 6H (Kleinhofs et al. in Matthews and Anderson 1994). Whether this locus is distinct from *XDhn3* or represents genes in the multigene locus *XDhn3*, which are more homologous with pTZ19R-dhn5 than pTZ19R-dhn3, is not clear.

Comparison of the partial clone pESI18 hybridization profiles in Southern blots of genomic DNAs of disomic substitutions of *L. elongatum* chromosomes in bread wheat with copy equivalents suggested that there was a single homologous gene corresponding to ESI18 and 4 or more closely related genes (Dubcovsky et al. 1994). Similar evidence was obtained for pTZ19R-dhn3 with bread wheat-barley disomic addition lines (Close and Chandler 1990). The DNA fragment hybridizing the most intensely with pTZ19R-dhn3 and 4 was from barley chromosome 6.

In the *Dra*I hybridization profile of *T. monococcum* genomic DNAs hybridized with pESI18-1; 4 intense and 1 faint fragments were polymorphic among the 14 observed fragments. The fragments with an intense signal cosegregated and were from *XDhn3* locus, but the fragment with the faint signal was from the *XDhn2.2* locus on $5A^m$. Since the remaining 9 DNA fragments could not be mapped, it is not known whether these DNA fragments are from the *XDhn3* locus or other loci. In chromosome arm mapping with the partial clone pESI18, ditelosomic addition and substitution lines involving chromosome arm 5EL showed no restriction fragment hybridizing with the probe; all restriction fragments were from chromosomes 6E, 6A, 6B, and 6D (Dubcovsky et al. 1994). If the homology between pESI18 and restriction fragments from the loci on the long arms of the *L. elongatum* and wheat chromosomes of homoeologous group 5 was as low as the homology found here between clone pESI18-1 and the restriction fragment from *XDhn2.2* locus of *T. monococcum*, it is likely that the signal from the homoeologous group 5 chromosomes was below the detection level in the 5EL disomic addition and disomic substitution lines because of hexaploidy. This possibility would be even more likely if the homology of the partial clone ESI18 with chromosome 5 restriction fragments would be lower than that of the full-length clone ESI18-1.

The nucleotide sequence of clone pESI18-1 has an overall nucleotide sequence similarity of 49% with the full-length clone pESI35-23; however, a conserved 42-bp motive in the 3′ region has a nucleotide sequence similarity greater than 75% (P. J. Gulick, personal communication). The clones did not hybridize to any common restriction fragment under the hybridization conditions used in this study. The truncated sequence of 39 amino acids at the C-terminal region of pESI18 showed 80% homology to barley dehydrin clone pTZ19R-dhn3 (Gulick and Dvořák 1992). Thus, genes in the paralogous *L. elongatum* loci *XEsi18* and *XEsi35* are more different from each other than are the genes at the orthologous *XDhn3* loci between *L. elongatum* and barley. Moreover, genes at the *XDhn3* locus are also more closely related to those at *XDhn2.2* and *XDhn6* than to *XEsi35*, which is indicated by the observation that DNA fragments at these loci hybridize with varying intensity with probes prepared from pTZ19R-dhn 1, 2, 3, 4, 5, and 6 and pTtu1937 (WSP16) (Table 1). There was no cross-hybridization of pESI35 with *T. monococcum* DNA fragments mapping at *XDhn2.2*, *XDhn3*, and *XDhn6*. These observations consistently suggest that *XEsi35* is not a member of the dehydrin gene family.

Chromosome 7

A locus hybridizing with clone pESI2, previously found to be on chromosome arms 7EL, 7AL, and 7BL (Dubcovsky et al. 1994), was mapped between *Xabc305* and *XAmy2*; both these markers are on chromosome arm 7HL (Kleinhofs et al. 1993). A locus encoding an isoform of the B subunit of V-ATPase, *XVAtp-B1*, that is different from that encoded by *XVAtp-B2*, was mapped by hybridization of the pHTB1 probe proximal to *XAmy2* in the long arm. Clone pHTB1 shares 81% of nucleotide sequence homology with clone pHTB2, which is complementary to locus *XVAtp-B2* on chromosome $6A^m$ (Berkelman et al. 1994). The two clones did not hybridize to any common restriction fragment. A locus encoding the A subunit of V-ATPase was found to be distal to *XEsi2*. The locus is between *Xabc305* and *Xwg380*.

Conclusions

The limited nucleotide sequence information that is available about the *Esi* genes in the 5L synteny group revealed no homology among the clones and to the partial clone pESI18 (Gulick and Dvořák 1990). The fact that these clones did not hybridize with common restriction fragments provides additional evidence suggesting that these cDNA clones correspond to unrelated genes (present data and Dubcovsky et al. 1994). The location of the 4 *Esi* genes at different chromosomal loci parallels these observations.

In contrast, dehydrin, pESI18, and pTtu1937(WSP16) clones correspond to genes that are part of a large multigene family located at at least 4 loci on three *T. monococcum* chromosomes. The dehydrin, pESI18, and pTtu1937(WSP16) clones share a lysine-rich motif that characterizes group 2 LEA, type (D-11), proteins (Close et al. 1993; Gulick and Dvořák 1992; King et al. 1992). This motif is found near the carboxy terminus of the proteins, and a variable numbers of these motifs are internally repeated (Close et al. 1993).

The distribution of the stress-related genes in the *T. monococcum* genome shows a relatively high number of stress-related genes on chromosome arm $5A^mL$. Whether this is simply sampling effect or a real phenomenon is not clear at this point.

The present paper illustrates the utility of mapping functionally related genes in a common linkage map for revealing their relationships. Since sufficient reserves of DNA and seeds of the *T. monococcum* DV92×G3116 mapping population are available, we propose to continue to use this mapping population for the investigation of the relationships among clones of other stress-related genes.

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