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BRIEF COMMUNICATION

Mapping of esterase loci in *Aegilops uniaristata* and homoeologous group 3 chromosomes of wheat

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Abstract

This study was planned to identify the chromosomal location of esterase loci in wheat (*Triticum aestivum*), in comparison to *Aegilops uniaristata*, using wheat *Ae. uniaristata* disomic addition and translocation lines. Two loci (Est-N1 and Est-N8) were identified on 3N chromosome of *Ae. uniaristata* and their probable homoeoloci were, for the first time, mapped close to three RFLP probes (Xpsr56, Xpsr394, and Xpsr1196) on homoeologous group 3 wheat chromosomes.

Additional key words: addition lines, esterase isozymes, RFLP, translocation line, *Triticum aestivum*.

Alien chromosome addition and translocation lines have been used to study homoeologous relationships (Iqbal *et al*. 2000a) for assigning genes on specific chromosomes (Delibes *et al*. 1981), and for the production of recombinants between alien chromosomes and those of the wheat (Iqbal *et al*. 2000b). *Aegilops* species are close relatives of wheat and are considered valuable source of important genes (Ceoloni *et al.* 1992). *Ae. uniaristata* is considered gene source for tolerance to stripe rust (Mikhova 1988), kernal bunt (Warham *et al*. 1986), cereal cyst nematodes (Dosba and Rivoal 1982), and aluminium (Berzonsky and Kimber 1986, Miller *et al*. 1995). Isozyme markers have been used for the identification of cultivars (Krulíčková *et al*. 2002), conformation of hybrids (Farooq *et al.* 1996) and to study genetic fidelity (Ramalakshmi Dutta *et al*. 2003/4). The use of biochemical markers can be of help both in the identification of addition lines and in the further manipulation of these lines in breeding programs. Presence of specific alien chromosome and/or chromosome segments can be confirmed by simple biochemical markers in successive generations of crosses.

Mostly addition lines are used to generate data about the chromosomes of alien species but the aim of the present study was to use *Ae. uniaristata* disomic addition and translocation lines for the identification and mapping of homoeoloci in wheat.

The plant material consisted of parental species *Triticum aestivum* L. cv. Chinese Spring (2n=6x=42, AABBDD), *Aegilops uniaristata* Vis. accession 2120001 (2n=2x=14, NN), six disomic addition lines of *Ae. uniaristata* in Chinese Spring (CS) background and one 3BL/3NL translocation line where long arm of chromosome 3N of *Aegilops uniaristata* was spontaneously translocated to the long arm of chromosome 3B of wheat. Addition lines were produced by direct backcrossing of Chinese spring × *Ae. uniaristata* F1 hybrid as colchicine doubling of the hybrid could not be achieved. The 6N addition line was not available and therefore could not be included in this study.

Enzyme extracts were prepared by grinding young green leaves from 20-d-old seedlings in Tris-Cl sample buffer (0.125 M Tris, 10 % glycerol), pH 6.8, in precooled mortar and pestles. Sample slurry was collected in eppendorf tubes, centrifuged for 15 min and 0.025 - 0.030 cm^3 supernatant was subjected to electrophoretic separation in 8 % non-denaturing discontinuous polyacrylamide gels following the methods of Laemmli

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Abbreviations: Est - esterase; RFLP - restriction fragment length polymorphism.

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(1970). After electrophoresis gels were stained histochemically and method described by Ainsworth *et al.* (1984) was used to visualize esterase loci.

Eight different esterase loci were detected in the profiles of *Ae. uniaristata* and five of these loci were polymorphic to wheat. The zymogram could be distinguished into two mobility zones, a high mobility and that of a low mobility. In *Ae. uniaristata* three bands, Est-N1, Est-N2, and Est-N3, were present in high mobility zone while five (Est-N4, Est-N5, Est-N6, Est-N7 and Est-N8) could be distinguished in the low mobility zone. In wheat also eight different bands, three in the low mobility and five in the high mobility zone, could be scored. Three bands of lowest mobility were common in both the species, *i.e*., Est-N6, Est-N7 and Est-N8 of *Ae. uniaristata* were monomorphic to wheat. The highest mobility band (Est-1N) was not present in the profiles of any of the addition lines used in this study. Esterases Est-N2 and Est-N3 appeared as one thick band in *Ae. uniaristata* but were actually two bands of almost same mobility, Est-N2 being slightly faster than Est-N3. Est-N2 is expressed in chromosome 2N addition line while Est-N3 by 3N addition line. Similar results were also observed for Est-N4 and Est-N5.

As the highest mobility locus (Est-N1) was not expressed in any of the studied addition lines it is, therefore, present on the chromosome 6N of *Ae. uniaristata*, the addition line that was unfortunately not available for study. A monomeric leaf specific isozyme locus (Est-4) has been described (Figueiras *et al.* 1986) on long arms of chromosomes 6B and 6D of wheat suggesting homoeology between these loci. The loci at positions two and four with respect to their mobility (Est-N2 and Est-N4) in *Ae. uniaristata* profile were also expressed by chromosome 2N addition line. Liu and Gale (1990) have described green tissue esterases (Est-7) in wheat and related species under the control of a set of genes on the long arms of chromosomes 2A, 2B and 2D. The loci expressed in 2N addition are two different loci and one of these loci could be homoeologus to the Est-7 locus of wheat. Keeping in view the mobility of these loci it may be suggested that the low mobility band Est-N4 is probably homoeologus to the wheat Est-7. The locus Est-N2 is specific to *Ae. uniaristata*.

The loci Est-N3 and Est-N5 were expressed in the profiles of chromosome 3N addition line. Liu and Gale (1990) have reported esterases (Est-1) that are expressed in all tissues except endosperm and are located on the short arms of group 3 chromosomes of wheat. In wheat another set of esterases (Est-8) have also been reported which are expressed in the vegetative tissues and leaves (Jouve and Diaz 1990, Liu and Gale 1990) and

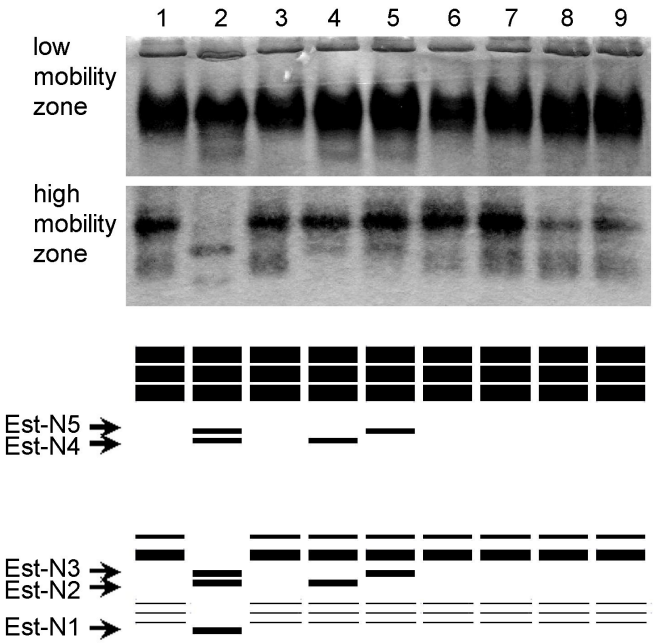

Fig. 1. Zymogram of esterase isozyme profiles of Chinese Spring (*lane 1*), *Aegilops uniaristata* (*lane 2*), 1N addition line (*lane 3*), 2N addition line (*lane 4*), 3N addition line (*lane 5*), 4N addition line (*lane 6*), 5N addition line (*lane 7*), 7N addition line (*lane 8*), and 3BL/3NL translocation line (*lane 9*). The highest mobility band (1N) is not present in any of the addition lines and therefore expressed by 6N chromosome of *Ae. uniaristata*. Esterase loci Est-N2 and Est-N4 are expressed by 2N addition line. Loci Est-N3 and Est-N5 are present in the 3N addition but are not expressed by 3BL/3NL translocation line. These two loci are therefore present on the short arm of chromosome 3N of *Ae. uniaristata*.

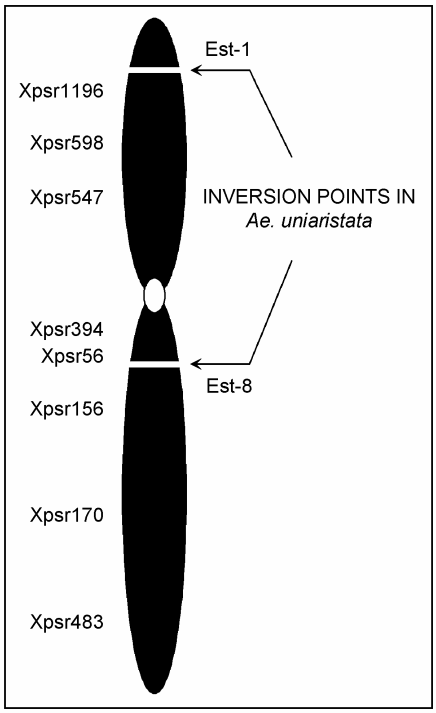

Fig. 2. The positions of RFLP probes on group 3 chromosomes of wheat, points of inversion in *Ae. uniaristata*, and the map positions of Est-1 and Est-8 isozyme loci.

are located on the long arms of group 3 chromosomes in wheat. Although these loci were expressed by 3N addition line there was no expression in 3BL/3NL translocation line indicating that in *Ae. uniaristata* both these loci are present on the short arm of chromosome 3N. Chromosome 3N of *Ae. uniaristata* carries an asymmetric pericentric inversion, with respect to wheat, in which part of the long arm has been transferred to the short arm while about 80 % of the short arm is a part of the long arm as result of this inversion (Iqbal *et al*. 2000a,b). From our findings and previous observations it can be deduced that locus Est-8 present on the long arm has been transferred on to the short arm of chromosome 3N while the position of the locus Est-1 on the short arm has not been effected by this inversion. Therefore, the genes for Est-8 are present close to the RFLP probes Xpsr56 and Xpsr394 on the long arms of homoeologous group 3 chromosomes of wheat because only these two RFLP loci were transferred to the short arm in *Ae. uniaristata* as a result of inversion. The position of Est-1 locus has not changed after the inversion indicating that the genes for this locus are present beyond the point of inversion and RFLP probe Xpsr1196 on the short arm of *Ae. uniaristata* and wheat group 3 chromosomes. Fig. 2 indicates the points of pericentric inversion (Iqbal *et al*. 2000a) and the mapped positions of the esterase loci on wheat group three chromosomes.

Although homoeology of three *Ae. uniaristata* esterase loci could not be determined from these studies position of two sets of esterase genes were, for the first, mapped close to the RFLP probes on homoeologous group 3 chromosomes of wheat.

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