Present progress in the characterization of *Triticum aestivum/Aegilops ventricosa* transfer lines

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A wide range of stable hexaploid lines (H-93) obtained by repeated selfing of the progeny from a cross (*T. turgidum* H-1-1 x *Ae. ventricosa* AP-1) x *T. aestivum* cv. Almatense H-10-15, has been previously studied. These lines (H93-1 to H-93-70) were found to carry genes from the D^v and the M^v genomes of *Ae. ventricosa*, which had been incorporated both by chromosomal substitution and by recombination. The incorporation of genes for resistance to eyespot (*Pch1*) and to powdery mildew (*Pm*) into wheat chromosomes has been demonstrated also. Recent progress in the study of the H-93 lines includes the following aspects: a) Association of gene *Pch1* in transfer line H-93-70 with a different chromosome than the resistance factor in "Roazon" wheat; b) possible correlation of a polymorphism in mt-DNA with a cytoplasmic effect on eyespot resistance; c) demonstration of a $4M^v$ substitution in transfer line H-93-33; d) application of the same breeding scheme to obtain gene transfer from *Ae. triuncialis*.

The transfer of genetic material from wild species to cultivated ones has been extensively exploited as a way to introduce certain agronomic traits, such as resistance to different diseases, into well adapted, highly productive cultivars. Previous work from this laboratory has demonstrated the effectiveness of a particular strategy to transfer genes from the wild grass *Aegilops ventricosa* to the cultivated wheat *Triticum aestivum* (Delibes & García Olmedo 1973, Delibes et al 1977a, Doussinault et al 1983, Delibes et al 1987). The strategy consists in crossing the donor species, *Ae. ventricosa* (genomes $D^vD^vM^dM^d$) with *T. turgidum* (AABB), which acts as a bridge, and rescuing the sterile ABD^vM^v hybrid with pollen from the recipient species *T. aestivum* (AABBDD). Plants resulting from this cross were fertile and after repeated selfing, stable lines with 42 chromosomes were derived from them (H93-1 to H-93-70). The H-93 lines were screened for biochemical markers encoded by genes located in the D^v and M^v genomes of *Ae. ventricosa* (Delibes et al 1973, 1977a,b). Due to the partial homology of the D^v genome of the donor and the D genome of the recipient, markers associated with the D^v genome appeared at high frequencies (30-60%). Genes from the M^v genome, which is not homologous to any of the hexaploid wheat genomes (A, B or D), generally appeared at lower frequencies (<4%), although at least one such gene did appear at a higher frequency (Delibes et al 1977b). A study of meiosis in hybrids of these lines and the recipient hexaploid wheat, together with an investigation of the distribution of biochemical chromosome markers, indicated that genes from the donor had been incorporated into the transfer lines both by chromosomal substitution and by recombination (Delibes & García-Olmedo 1973, Delibes et al 1977b, García-Olmedo et al 1984). The incorporation of genes for resistance to eyespot disease (*Pch1*), caused by the fungus *Pseudocercosporella herpotrichoides*, and to powdery mildew (*Pm*), caused by the fungus *Erysiphe graminis*, into recombinant wheat chromosomes, following the above strategy, has been demonstrated (Doussinault et al 1983, Delibes et al 1987a). We now report on our progress in the characterization of the H-93 lines. More specifically, the following aspects

will be discussed: a) association of gene *Pch1* in transfer line H-93-70 with a different chromosome than the resistance factor in Roazon wheat; b) possible correlation of a polymorphism in mt-DNA with the different contribution of the cytoplasms of *T. turgidum* H-1-1 and *T. aestivum* cv. Almatense H-10-15 to the resistance to eyespot; c) demonstration that line H-93-33 carries a $4M^v$ chromosome; d) use of the above-described breeding scheme to transfer genetic material from *Ae. triuncialis* to hexaploid wheat.

MATERIALS AND METHODS

T. aestivum cv. Almatense H-10-15, *T. turgidum* H-1-1, and *Ae. ventricosa* AP-1, and lines H-93-1 to H-93-70, derived from them, have been previously described. Wheat-*Agropyron* 7D/*7Ag* substitution line was the gift of E.R. Sears (Columbia, Mo. USA). *T. aestivum* cv. Pané 247 has the sterol-esters pattern corresponding to the recessive allele *pln* (Palmitate negative; García-Olmedo 1968). *Aegilops triuncialis* AP-1 was from the collection of M. Alonso Peña.

Resistance to eyespot was tested as previously described (Doussinault et al 1983). Sterol esters were analysed according to García-Olmedo (1968), and proteins NGE-11 and C7 by a modification of the method of Rodriguez-Loperena et al (1975). Isozymes of phosphatase and of alcohol dehydrogenase were separated by standard procedures (Delibes et al 1977b, 1981).

Chloroplast DNA was prepared according to Bogorad et al (1983) and mitochondrial DNA as in McNay et al (1983), followed by purification in CsClbis-benzimide gradient (De Bonte & Mathews 1984). Protein synthesis by isolated mitochondria was investigated by the procedures described by Forde et al (1979).

RESULTS AND DISCUSSION

1. Gene *Pch1* is not on chromosome 7D

In transfer line H-93-70, the gene for eyespot resistance *Pch1* has been integrated into a wheat chromosome, as judged from the regularity of the meiosis of the hybrid between this line and the recipient hexaploid wheat, *T. aestivum* cv. Almatense H-10-15 (Delibes et al 1977b, Doussinault et al 1983). A resistance factor in Roazon wheat, which had been transferred from line VPM1, has been found to be associated with chromosome 7D by F2 monosomic analysis (Jahier et al 1979).

To investigate if gene *Pch1* was also associated with chromosome 7D, crosses of the resistant line H-93-70 with the susceptible wheat cv. Pané 247 and with a 7D/*7Ag* wheat/Agropyron substitution line were carried out and F2 kernels were obtained. The kernels were cut transversally and the halves carrying the embryos were used for the resistance test, while the distal halves were used for genetic typing, based on the distribution of appropriate biochemical markers.

In the crosses involving cv. Pané 247, resistance was found not to be associated with the 7D locus *Pln*, which determines a sterol ester pattern with palmitate (dominant allele in H-93-70) or without palmitate (recessive allele in Pané 247).

In the crosses with the 7D/*7Ag* substitution line, resistance was neither associated with protein NGE-11 (7D marker), nor alternatively inherited with respect to protein C-7 (*7Ag* marker). Since no recombination has been observed between chromosomes 7D and 7Ag (Sears 1977), it is concluded that gene *Pch1* represents a different locus than the resistance factor in Roazon wheat, which has an independent origin. A more detailed account of these results has been submitted elsewhere.

2. Possible molecular basis of a cytoplasmic effect on eyespot resistance

The proportion of plants in which black mycelium of *Pseudocercosporella herpotrichoides* can be detected after infection is significantly higher when the cytoplasmic genetic determinants (chloroplast and mitochondrial genomes) are from the hexaploid *T. aestivum* cv. Almatense H-10-15 than when these were from the tetraploid *T. turgidum* H-1-1, the bridge species used in the transfer of

the nuclear resistance gene *Pch1* from *Ae. ventricosa* to hexaploid wheat (Delibes & García-Olmedo 1973, Delibes et al 1977a, Doussinault et al 1983).

To investigate the possible molecular basis for the different performance of the two cytoplasms with respect to eyespot resistance, a comparison of chloroplast and mitochondrial DNAs was carried out. No difference in the chloroplast DNA from the two sources after digestion with the restriciton endonucleases *Bam*HI, *Hind*III, *Pst*I, *Pvu*I and *Sal*I was found.

A 10.5 kb DNA fragment was present in the *Hind*III digest of mitochondrial DNAs from *T. turgidum* H-1-1 and from the resistant line H-93-70, which was absent from the *Hind*III digest of the mt-DNA from *T. aestivum* cv. Almatense H-10-15, the recipient species in the gene transfer experiment.

To check if the difference observed at the DNA level affected any of the proteins encoded by the mitochondria, protein synthesis by isolated mitochondria was investigated. No differences in the electrophoretic patterns of proteins synthesised were detected. Characterisation of the 10.5 kb fragment is in progress.

3. A 4M^v chromosome substitution in line H-93-33

Biochemical characters present in *Ae. ventricosa* (D^{v}M^v), *Ae. comosa* (M), and *Ae. uniaristata* (M^u), and absent in *T. aestivum* (ABD), *Ae. squarrosa* (D) and *T. turgidum* (AB), have been selected as possible markers of chromosomes from the M^v genome (Delibes & García-Olmedo 1973, Delibes et al 1977b). Two such markers, Aph-v (phosphatase isozyme) and protein CM4 (equivalent to NGE-17v) have been previously reported as present in transfer line H-93-33 (García-Olmedo et al 1984). The first marker appeared in the H93 lines with the low frequency expected of M^v genome markers, whereas CM4 was present in a high proportion of the lines (31%) and appeared alternatively with protein NGE-17, a marker of chromosome 4D (Delibes et al 1977a). In a separate study concerning *Ae. ventricosa* addition lines obtained from a different accession from that used to derive the H-93 lines, both markers appeared associated with the same M^v chromosome (Delibes et al 1981). This, and the previous assignment of phosphatase isozymes to group 4 chromosomes (Brewer et al 1969) led to the tentative identification of the chromosome carrying these markers as 4M^v (Delibes et al 1981). Using both isozyme staining and DNA hybridisation techniques, we have now demonstrated that a third marker, a variant of alcohol dehydrogenase (Adh-μ) is present both in line H-93-33 and in the putative 4M^v addition line. The Adh system has also been associated with group 4 chromosomes in wheat (Hart 1970). Linkage of the three markers under study in line H-93-33 was confirmed through the appropriate crosses with *T. aestivum* cv. Almatense H-10-15 and the biochemical analyses of F2 kernels. This finding further supported the tentative identification of the 4M^v addition line (Dosba 1985) and strongly suggested a 4M^v substitution in the H-93-33 transfer line. To confirm these conclusions, cytological studies of hybrids between line H-93-33 and the appropriate ditelosomics have been carried out.

4. Application of the same gene-transfer strategy to *Ae. triuncialis* introgression

Aegilops triuncialis (CCUU) has been crossed with the tetraploid wheat *T. turgidum* (AABB) and the resulting ABCU sterile hybrid has been rescued with pollen from the hexaploid *T. aestivum* (AABBDD). Seven spikes from two hybrid plants were pollinated with pollen from cv. Almatense H-10-15 and 8 kernels were obtained. Low fertility (3-5 viable kernels per plant) and high vigour were observed throughout the process. Further crosses to *T. aestivum* will be performed to obtain stable, fertile lines and these will be screened for *Ae. triuncialis* genetic material with the aid of previously identified biochemical markers and recently developed DNA probes.

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