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Chromosomal location in wheat of the genes coding for the acyl carrier proteins I and III

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Source of the probes

A cDNA library in λgt 11 was constructed from poly(A^+) RNA, isolated from barley seedling leaves. The library was screened with a 66-bp oligonucleotide probe, constructed on the basis of the cDNA sequence of ACP I of spinach and the highly conserved region of ACP I of barley. Inserts from 22 positive plaques were isolated and subcloned into the EcoRI site of pUC18. Sequencing demonstrated that 19 out of the 22 clones coded for the chloroplastic acyl carrier protein I (ACP I). The 3 other clones coded for the acyl carrier protein III (ACP III), which is thought to be achloroplastic. No clones were obtained for the chloroplastic acyl carrier protein II (ACP II). Two clones were selected as probes for the gene localization: pACP11, with an insert of 768 bp coding for ACP I, and pACP1, with an insert of 520 bp coding for ACP III (Hansen 1987; von Wettstein-Knowles 1989).

Chromosomal location

pACP11 (Acl1)

Hybridization of pACP11 against four restriction digests of 21 nullisomic-tetrasomic and 24 ditelosomic lines of Chinese Spring (CS) wheat showed the gene, designated *Acl1*, to be located on the short arms of chromosomes *5A*, *5B* and *5D*. Since only one band was detected on each of the group 5 chromosomes, we conclude that only one copy of *Acl1* is present in each genome. This corresponds with the previously obtained location of the *Acl1* gene on *5H* (barley chromosome 7) (von Wettstein-Knowles 1989).

Table 1 shows the fragments associated with each of the three loci in CS, cut with four restriction enzymes, and the Betzes locus, cut with three restriction enzymes.

Table 1. Fragment sizes (kb) and number of alleles of the A, B, and D genomes of Chinese Spring wheat and the H genome of Betzes barley with different restriction enzymes

	EcoRI	EcoRV	HindIII	DraI
pACP11 (*Acl1*)				
5A	15.2 (1)[1]	12.1 (1)	2.6 & 4.6 (1)	3.7 (1)
5B	12.7 (1)	10.4 (1)	10.9 (1)	3.0 & 4.2 (1)
5D	8.0 (2)	4.6 & 9.7 (1)	2.8 (1)	1.9 (1)
7H	11.4 (1)	1.8, 4.5 & 8.9 (2)	6.2 (2)	
pACP1 (*Acl3*)				
7A	5.3 (2)	6.2 (2)	2.8 (2)	1.9 (2)
7B	5.0 (1)	9.8 (1)	6.5 & 7.7 (2)	26.4 (1)
7D	6.0 (3)	5.1 (1)	2.7 & 3.7 (2)	15.5 (2)
5B	6.8 (1)	3.4 (1)	4.7 (1)	4.8 (1)
7H	15.4 (2)	15.7 (2)	2.8 & 3.4 (2)	

[1] Number in parenthesis ( ) indicates the number of alleles

pACP1 (Acl3)

The hybridization patterns obtained with the pACP1 probe in four different restriction digests of CS consist of four bands (Table 1). Considering the hexaploid nature of Chinese Spring wheat, this suggests the presence of an additional copy of the gene on one of the chromosomes. This was confirmed by nullisomic-tetrasomic and ditelosomic analyses, which showed the genes, designated *Acl3*, to be located on the short arms of chromosomes *7A*, *7B* and *7D*, with a further copy on the long arm of chromosome *5B*. The chromosomal locations can be deduced both by the absence of hybridizing bands in the critical genotypes and by the increased hybridization in the corresponding tetrasomics (Fig. 1). Previous Southern blot analysis using Chinese Spring wheat-Betzes bar-

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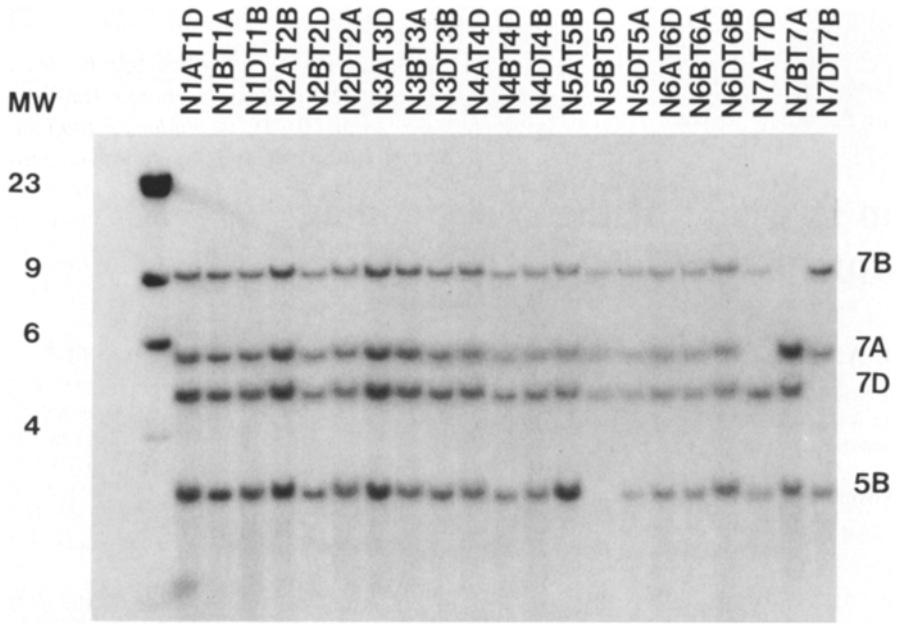

Fig. 1. Autoradiograph showing hybridization patterns obtained from an EcoRV digest of a complete set of Chinese Spring nullisomic-tetrasomic lines probed with pACP1 (*Acl3*)

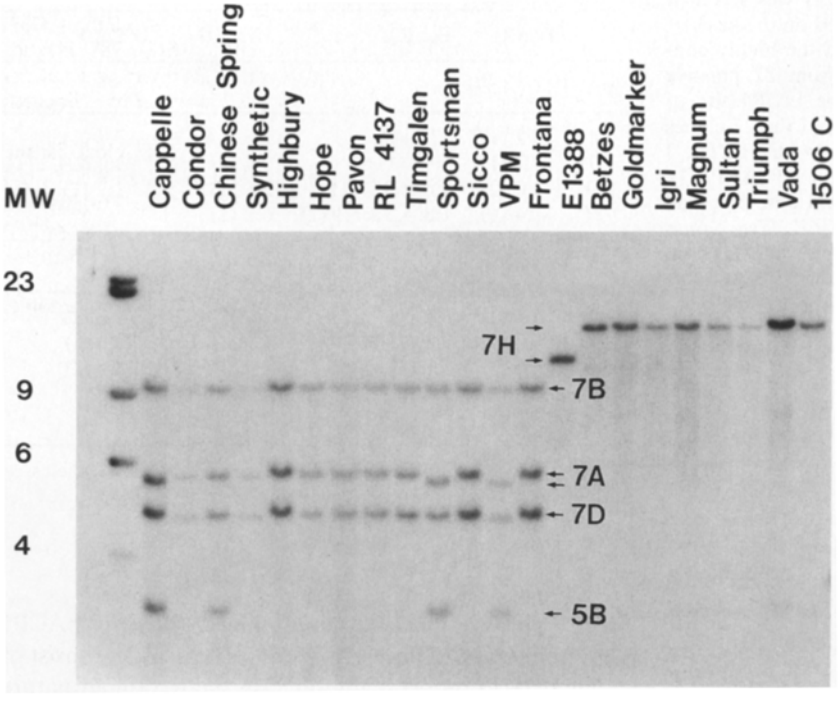

Fig. 2. Autoradiograph showing hybridization patterns obtained from an EcoRV digest of 13 wheat and 9 barley varieties probed with pACP1 (*Acl3*)

ley addition lines had already assigned the *Acl3* gene to *7H* (barley chromosome 1) (von Wettstein-Knowles 1989). Only a single hybridizing band was obtained, indicating that only one copy of the *Acl3* gene is present in Betzes barley.

Locus symbols

XAcl1 (*XAcl1-5A*, *-5B*, *-5D*).
XAcl3 (*XAcl3-7A*, *-7B*, *-7D*, *-5B*).

Polymorphisms

pACP11 (Acl1)

No polymorphism was found for the loci on *5A* and *5B* among four restriction digests, using EcoRI, EcoRV, HindIII and DraI, of 13 wheat varieties. For the *XAcl1-5D* locus, two alleles and a potential heterozygosity (H), assessed from all possible pairwise varietal comparisons (Crow 1986), of 26.0% were found, but only in EcoRI digests.

A comparably low value of H of 14.2% was found with two out of three restriction enzymes, EcoRV and HindIII, among the 13 barley varieties tested. With these enzymes, only one variety differed from the other 12. The other enzyme, EcoRI, revealed no variation.

pACP1 (Acl3)

The *XAcl3-7A* locus is moderately polymorphic with all four restriction enzymes. Two alleles and an average value of H of 33.1% were obtained. The degree of polymorphism of the *7B* and *7D* loci varied considerably with the enzyme used. For the *7B* locus, only HindIII showed variation, with two alleles ($H=42.6\%$). Two alleles ($H=14.2\%$) were observed at *XAcl3-7D* with HindIII and DraI, while three alleles ($H=27.2\%$) were found with EcoRI. No polymorphism was detected with EcoRV.

The extra copy of ACP III on chromosome *5B* shows an interesting RFLP pattern among the 13 wheat varieties shown in Fig. 2. Three other varieties, besides CS, show the same *XAcl3-5B*-EcoRV band, but the remaining nine varieties show an apparent 'null' phenotype ($H=42.6\%$). Similar patterns were observed with the other three restriction enzymes. This confirms that the phenotype of these latter varieties really is null, rather than being a fragment that comigrates with one from the group 7 loci, and that the polymorphism is 'on-off' rather than a fragment length difference.

In barley, the *XAcl3-7H* locus is moderately polymorphic with all three enzymes. Two alleles and an average value of H of 22.1% were obtained (Fig. 2).

Other studies of acyl carrier protein genes

Reports of characterization of *Acl* genes for *Brassica campestris* (Rose et al. 1987), *Brassica napus* (Safford et al. 1988; de Silva et al. 1990), *Arabidopsis thaliana* (Post-Beittenmiller et al. 1989) and *Spinacea oleacea* (Scherer and Knauf 1987) are available.

Probe availability

Contact P. von Wettstein-Knowles.

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