OBSERVATION OF LINKAGE BETWEEN Wsp AND THE ISOZYME LOCUS Alat-p

Weeden, N. F. NYS Agricultural Experiment Station, Geneva, MY USA

The current linkage map (2) places the waxless mutant, wsp, on one end of the chromosome 7 linkage group; however, veritication of this location has been difficult (G. A. Marx, pers. commun.). In my own work I have been unable to find linkage between Wsp and the loci Tl, K, Est-4, or Bt, all believed to be on chromosome 7. In this communication I present evidence for linkage between Wsp and the isozyme locus Alat-p, which codes for the plastid-specific form of alanine aminotransferase (ALAT-1).

An F2 population from the cross A778-26 x A73-91 was analyzed for segregation at both Wsp and Alat-p. A778-26 was homozygous dominant for Bt, Wsp, and R and possessed the fast allozyme of ALAT-1. A73-91 was Wellensiek's tester (bt, wsp, r and displayed the slow allozyme of ALAT-1). Both lines were obtained from Dr. G.A. Marx. The ALAT-1 phenotype could be determined in young leaf extracts by horizontal starch gel electrophoresis using the buffer system consisting ot 50 mM Trizma base (Sigma) neutralized to pH 8.0 with L-histidine-HCl as the electrode buffer and a 3.5 mM Trizma/histidine pH 8.0 buffer in the gel. The alanine aminotransferase assay contained 10 ml Tris-HCl pH 8.0, 20 mg L-alanine, 10 mg alpha-ketoglutarate, 15 mg NADH, 3 mg pyridoxal-5-phosphate and 200 units of lactate dehydrogenase. The assay mixture was poured on to the cut surface of the gel and allowed to sit tor 10 min. The excess assay solution was poured off the gel and the gel incubated for 10-20 min at 20-30 C. The ALAT activity could be observed as dark bands against a bright fluorescent background when viewing the gel under ultraviolet light (302 nm).

Variation was present for ALAT-1 but not for the more intensely staining, cytosolic ALAT-2. Both Wsp and Alat-p segregated as monogenically inherited characters, with the alleles of Alat-p showing codominant expression (Table 1). Joint segregation analysis indicated that the two loci were linked, being separated by about 16 recombinant units (Table 2). These results suggest that Alat-p might be useful in confirming the map location of Wsp. However, Alat-p did not show linkage with Tl, K, 6-pgd-p or Pep-4 The last two loci are linked to Rrn-2, the ribosomal RNA gene cluster presumably located on chromosome 7 (2). Alat-p also assorted independently of St, Le, Td, D, B, Fa, A, Gp, PI, Aat-m, Acp-1, and 6pgd-c (data not presented). Thus, at present, the precise location of Alat-p and Wsp remains uncertain.

1. Blixt, S. 1974. In: Handbook of Genetics, Vol. 2. K. C. King (ed.) Plenum Press, NY p. 181-221.
2. Polans, N. O., N. F. Weeden, and W. F. Thompson. 19«b. Theor. Appl. Genet. 72:289-295.

Table 1. Segregation at WSP and Alat-p.

	No. plants with designated phenotype*			Expected	
Locus	fast/+	H	slow/+	ratio	χ^2
WSP	62		18	3:1	0.26
Alat-p	20	43	17	1:2:1	0.68

*Designations: dominant phenotype or homozygous fast = +; heterozygous = -. recessive phenotype or homozygous slow = -.

Table 2. Joint segregation of Ws,p and Alat-p.

	No of plants with designated Phenotype*							Recomb.	
N	+/+	+/H	+/-	-/+	-/H	-/-	χ^2	Fraction	SE
80	19	33	5	1	5	12	29.0	16	4

*Designations as described in Table 1.

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