Location in linkage group III of a gene coding minor vicilin polypeptide

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The pea seed storage protein vicilin has M_r about 150 kD and consists of three primary subunits of M_r 50 kD. Sodium dodecylsulphate gel electrophoresis separates vicilin subunits into polypeptides of M_r 12.5, 13.5, 16, 19, 33, 35 and 50 kD (4). The initial products of the vicilin structural genes are polypeptides of M_r 50 and 47 kD (1). All of the 47 kD and some of the 50 kD precursors are proteolytically cleft yielding vicilin polypeptides of M_r<35 kD. There are two potential cleavage sites in vicilin precursors. Processing could take place at one point, at another, at both points or not at all if asparagine residues are absent in required positions (8). Taking into consideration that vicilin is encoded by several gene families (2), the observed variety of vicilin polypeptides should be expected.

It has been shown (5) that one of the genes coding the 50 kD vicilin subunit, *Vc-1,* is linked to the locus *r*. It also has been demonstrated that vicilin polypeptides of M_r 33 kD are controlled by a pair of co-dominant genes at a single locus (7). Further investigations were carried out using the length polymorphism of DNA sequences homologous to the vicilin messenger RNAs. Gene *Vc-2* was mapped on linkage group V and genes *Vc-3* and *Vc-5* on opposite ends of linkage group III (3, 9).

We have studied one of the minor vicilin polypeptides with an M_r about 35 kD which binds the vicilin antibodies in Western blot analysis. This polypeptide was designated by us Vc-mp. In dodecylsulphate gel electrophoresis Vc-mp has at least two allelic variants of M_r 35.4 (F) and 35.9 (S) kD (Fig. 1). Among 100 samples of the world pea collection at St Petersburg these variants were found with frequencies 0.58 and 0.38, respectively. Sometimes we encountered varieties lacking polypeptides in the zone of 35.4 or 35.9 kD.

We crossed lines VIR4907 (*Pisum sativum* ssp. *asiaticum,* Vc-mp S) and WL1238 (Vc-mp F). Analysis of F_1 seeds from the reciprocal cross VIR4907 x WL1238 showed co-dominant inheritance of the Vc-mp polypeptide patterns. This indicates that the Vc-mp polypeptide variants are due to variation in the nuclear structural genes and that there is no maternal effect on Vc-mp polypeptide inheritance. 156 F_2 seeds were examined. The observed segregation into Vc-mp classes was 37 SS: 81 SF: 38 FF [χ^2 (1:2:1) = 0.24], indicating monogenic control for the Vc-mp polypeptide synthesis. F_2 segregation data for the Vc-mp polypeptide and various morphological markers revealed significant linkage (P<0.0001) between the gene coding for the Vc-mp polypeptide and gene *b* in linkage group III (Table 1). The corresponding distance was calculated as 12.3 ± 2.8 map units. There was no evidence of linkage between the vicilin gene and any of the other markers (*d, f, gp, i, k, le, m, pi, r, s, tl, u* and *wb*) segregating in this cross. To locate the vicilin gene more accurately, we crossed lines VIR4907 (Vc-mp slow, *St, B*) and WL1749 (Vc-mp fast, *st, b*) differing in two marker genes of linkage group III. F_2 segregation data are shown in Table 1. The recombination fractions calculated from the data in Table 1 suggest the following arrangement of the genes:

st — 29 — 'Vc-mp' — 9 — *b*

As was mentioned above, two vicilin loci are already mapped on pea linkage group III. Vc-5 is situated approximately 25 map units from gene *st* in the opposite direction to gene *b* (3, 6). Thus *Vc-5* is in a different location to the gene coding for the Vc-mp polypeptide. *Vc-3* was placed on the "M end" of group III by Weeden *et al* (9) but it is now accepted that *Vc-3* lies below *st* in the general vicinity of the *b* locus (3, N.F. Weeden pers. com.). Thus *Vc-3* is very likely the gene coding for Vc-mp polypeptide. The map position 9 units above *b* is consistent with that conclusion but further work is necessary to fully resolve the identity of the vicilin gene mapped in our study.

Table 1. Dihybrid segregation data obtained from F_2 populations of crosses 1) VIR4907 (*B* Vc-mp slow) x WL1238 (*b* Vc-mp fast) and 2) VIR4907 (*B St* Vc-mp slow) x WL1749 (*b st* Vc-mp fast).

Loci		Cross	Phenotype[1]						Total	Joint χ^2	Recomb frac	SE
			DS	DH	DF	RS	RH	RF				
b	Vc-mp	1	36	71	7	1	10	31	156	77.48*	12.27	2.78
b	Vc-mp	2	45	87	4	0	8	40	184	127.14*	6.57	1.88
b	Vc-mp	1+2	81	158	11	1	18	71	340	202.97*	9.18	1.63
st	Vc-mp	2	41	71	20	4	24	24	184	23.74*	29.38	3.88
			DD	DR	RD	RR						
st	*b*	2	108	24	28	24			184	15.14*	32.23	6.51

[1] D - dominant, R = recessive, S = homozygous slow, H = heterozygous fast/slow, and F = homozygous fast The first named locus is shown first.
* P<0.0001

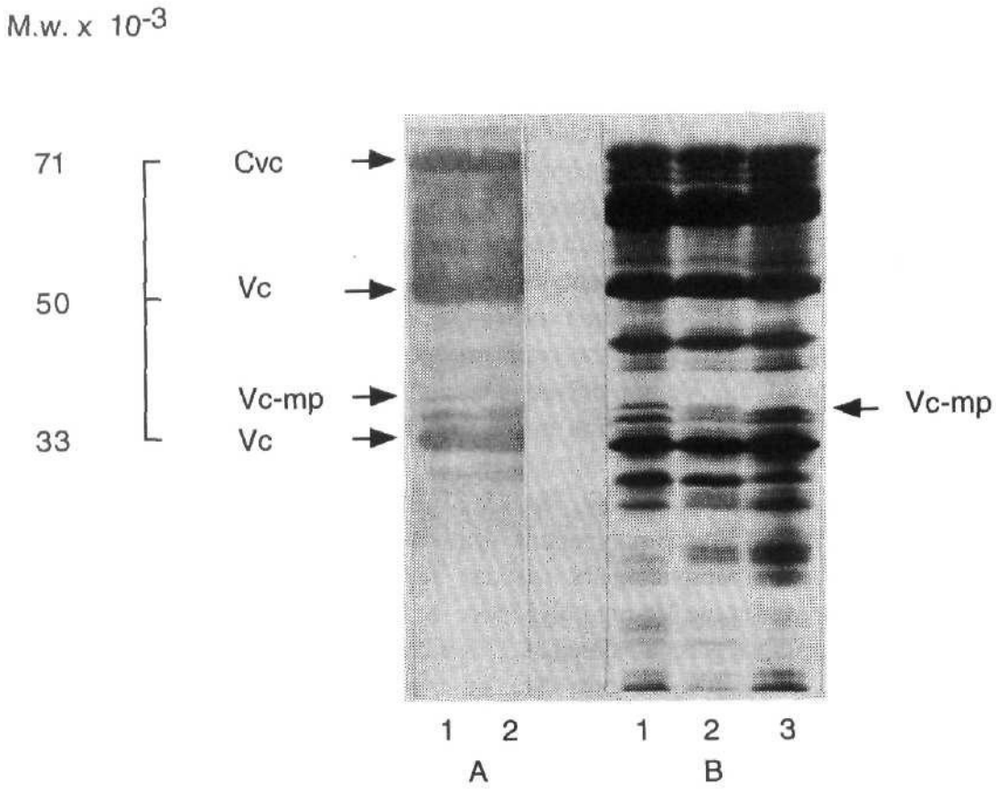

Fig. 1. Sodium dodecylsulphate polyacrylamide gel electrophoresis (non-reducing conditions) of pea seed proteins:
A - vicilin subunits, identified by "Western" blot analysis;
B - total protein extracts of seeds of parental lines VIR4907 (1) and WL1749 (3) and the F_1 (2) from their cross, stained by Coomassie R-250.

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