MAPPING OF A LEGUMIN SUBUNIT GENE ON PEA CHROMOSOME 7

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The major pea seed storage protein legumin contains six subunit pairs. They may be divided into three types: conventional (M_r 53000-54000), big (M_r 55000-58000) and small (M_r 35000), referred to as Lg-1, Lg-2 and Lg-3, respectively (6). Each subunit pair consists of α - and β-subunits, joined by disulphide bonds. The majority of legumin is synthesized as precursor molecules, containing covalently linked α- and β-subunits (2,5). So the genetics of β-subunits should follow those of α-subunits.

It has been reported that Lg-1 α-subunits are encoded in a set of closely linked genes at a single locus on chromosome 7, located approximately 7 map units (3) or 17 map units (6) from r in the direction of tl. The genetics of other legumin subunits has not been studied in detail. Lg-2 α-subunits segregated independently from Lg-1 α-subunits in one cross and were linked with them in the other. Linkage was found between Lg-2 α- and Lg-3 β-subunits, but independent segregation for Lg-3 α- and Lg-3 β-subunits was suggested (6).

Three classes of legumin cDNA were identified (4). One class of legumin genes showed linkage to gene r and corresponds to the Lg-1 α-subunits. A second class has been mapped to a locus near a on chromosome 1. A third class of legumin genes also appears to be linked with gene a (4). The relationship between the two last classes of legumin genes and particular α- and β-subunits is unknown.

Analysis of the seed proteins of pea line VIR319 (ssp. sativum) by sodium dodecylsulphate gel electrophoresis revealed a legumin subunit pair which could not be identified with any known subunit pair. This polypeptide of uncertain identity is symbolized temporarily as Lg-u. The M_r of Lg-u is approximately 3000 less than the M_r of Lg-1 (Fig. 1, non-reducing conditions). The difference between Lg-u and Lg-1 subunit pairs is due to their α-subunits, while the β-subunits of these pairs seemed to be identical (Fig. 1, reducing conditions).

Line VIR319 (Lg-u^+, present) was crossed with line WL1238 (Lg-u^-, absent). In F_1 hybrid seed Lg-u was expressed in a lesser amount (Lg-$u^{+/-}$) than in parent VIR319. An analysis of F_2 progeny (n=196) revealed segregation of Lg-u α-subunit classes + : +/- : - as 47 : 102 : 47, respectively. These results indicate Lg-u^+ and Lg-u^- to be a single allele pair (χ^2_1 = 0.33). Segregation of morphological markers and Lg-u α-subunits in F_2 plants showed linkage of Lg-u with genes r and tl (Table 1A). 13 plants showed recombination between genes r and tl, 32 between tl and Lg-u, and 45 between r and Lg-u. Corresponding distances were calculated as 3.3 ± 0.9, 8.2 ± 1.4 and 11.5 ± 1.6 map units, respectively, which generates the following map:

r--3.3--tl----------8.2 ----------Lg-u

Mapping of gene Lg-u showed that it is located in the same region on chromosome 7 as gene His-1, coding for histone H1 the slowest fraction. The distance between His-1 and tl was found to be 7.6 crossover units (7) while the data above indicate a distance of 8.2 units for Lg-u and tl. These

results were obtained in different crosses and the question arises as to which gene, Lg-u or His-1, is situated closer to gene tl. To answer this question I crossed lines 319.1 and Sprint, which differ not only in molecular variants of legumin and histone H1 the slowest fraction, but also in genes r and tl at one side of Lg-u and His-1, and gene Sa-K9 at the other side. Line 319.1 has genotype r, tl^w, His-1 F, Sa-K9 S, $Lg\text{-}u^+$ and line Sprint has genotype R_, Tl, His-1 S, Sa-K9 F, $Lg\text{-}u^-$. Only two F_2 plants among 132 had a recombinant phenotype in respect of genes Lg-u and His-1 (Table 1B). Thus, the map distance between Lg-u and His-1 is about 0.8 ± 0.5 map units. Recombinant plants had genotypes Rr $Tltl^w$ Lg-u+/- His-1 S, Sa-K9 F and Rr $Tltl^w$ Lg-u+/- His-1 F, Sa-K9 S.
These results indicate the following gene order:

r — tl — Lg-u — His-1 — Sa-K9.

The location of gene Lg-u on chromosome 7 coincides with that of Lg-1. Possibly, Lg-u is a member of the Lg-1 gene family and the Lg-u polypeptide may be the result of a deletion in one of the genes of this family. Several variant forms of the Lg-1 polypeptide have been described (1,6,8). Alternatively, Lg-u may be structurally different from the other major legumin polypeptides. Further study, e.g. sequencing or peptide mapping, is necessary to resolve these alternatives.

Table 1. Distribution of F_2 plants into phenotypic classes.

Gene pair		Genotypes									Chi-square	Recomb. fract.	SE
X	Y	XXYY	XXYy	XXyy	XxYY	XxYy	Xxyy	xxYY	xxYy	xxyy			
A. Cross VIR319 x WL12138, n = 196													
Lg-u	R	33	13	0	14	81	10	0	8	37	156	11.5	1.6
Lg-u	Tl	38	9	0	9	86	7	0	7	40	189	8.2	1.4
R	Tl	42	4	0	5	97	3	0	1	44	300	3.3	0.9
Expected		12.3	24.5	12.3	24.5	49	24.5	12.3	24.5	12.3	-	-	-
B. Cross 319.1 x Sprint, n = 132													
Lg-u	His-1	28	0	0	1	71	1	0	0	31	232	0.8	0.5
Tl	Lg-u	24	9	0	4	55	6	0	9	25	116	10.6	1.9
Tl	His-1	24	9	0	5	53	7	0	9	25	111	11.4	2.0
Tl	Sa-K9	23	9	1	9	46	10	0	12	22	80	15.9	2.2
His-1	Sa-K9	27	2	0	5	63	3	0	2	30	188	4.5	1.3
Expected		8.3	16.5	8.3	16.5	33	16.5	8.3	16.5	8.3	-	-	-

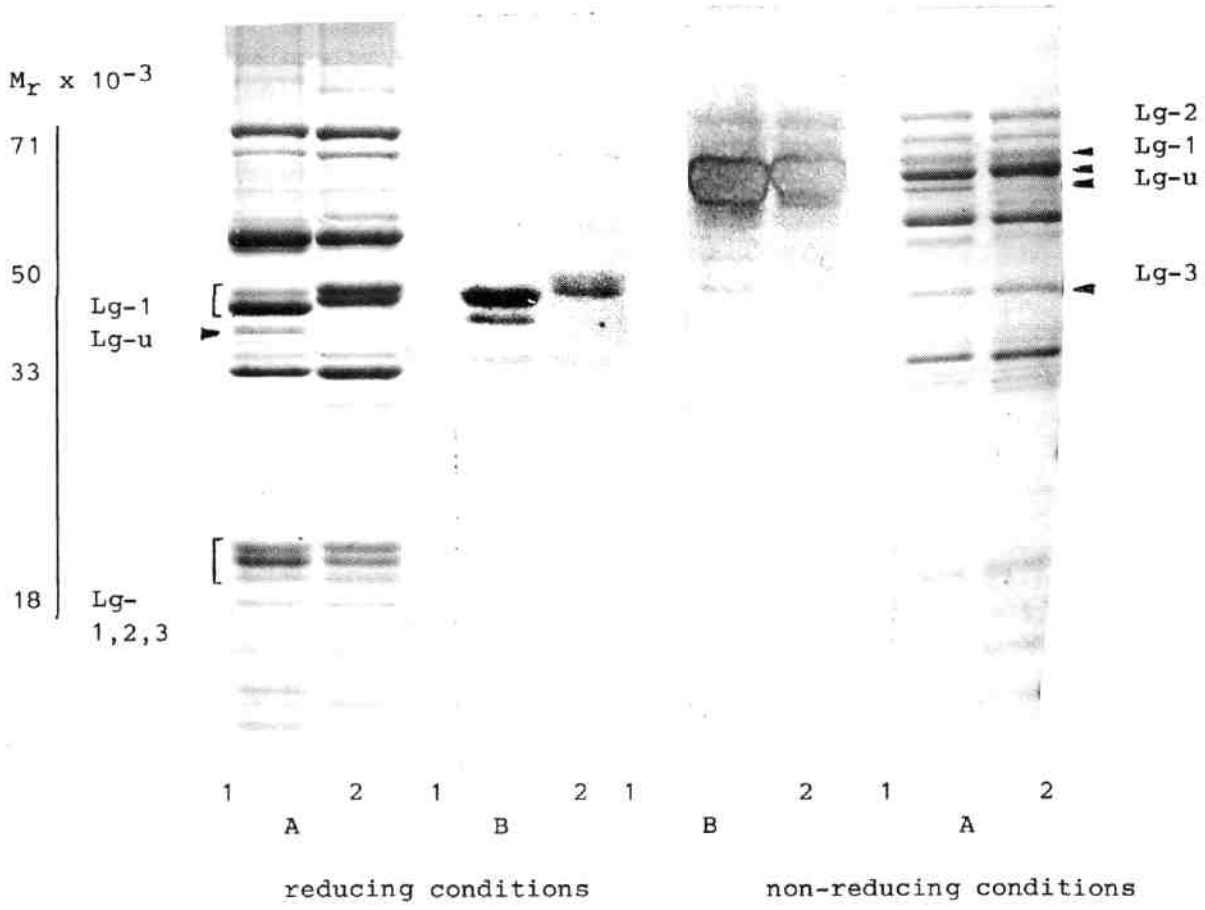

Fig. 1. Sodium dodecylsulphate polyacrylaramide gel electrophoresis of pea seed proteins in lines VIR319 (1) and WL1238 (2).
A - total protein extracts stained by Coomassie R-250,
B - legumin subunits (reducing conditions) and subunit pairs (non-reducing conditions) identified by "Western" blot analysis.

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