INHERITANCE AND MAPPING OF VICILIN STORAGE PROTEIN GENES IN *PISUM SATIVUM* L

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SUMMARY

Gel electrophoresis techniques have been used to investigate genetically controlled variation in the major subunits (Mr 50,000) of vicilin, a storage protein of *Pisum sativum* L. The F₁ protein band patterns were shown to be additive with respect to those of the parental lines and to be identical in reciprocal crosses. Genetic analysis of the F₂ plants indicated that the 50,000 Mr vicilin subunit band pattern is controlled by a pair of co-dominant genes at single locus. The F₂ data were used to locate this major vicilin gene locus (Vc-1) to chromosome 7, closely linked to the *r* locus (for round and wrinkled seed surface).

1. INTRODUCTION

Genetic variation in band patterns of the seed proteins of pea (*Pisum sativum* L.) on gel electrophoresis has been observed for both the major storage proteins, vicilin (Hynes, 1968) and legumin (Davies, 1973). In the case of vicilin, it was shown that F₁ seed protein band patterns arose from the additive patterns of the two parental types (*i.e.*, that vicilin protein molecules were inherited additively) and that the patterns were controlled by alternative alleles at a single locus. Further vicilin loci were identified by Thomson and Schroeder (1978) by carrying out gel electrophoresis under differing conditions, and the use of denaturing conditions allowed some of the vicilin loci to be identified with the “subunits” or component polypeptides of the protein. These results showed that in reciprocal crosses between two lines of peas, band patterns of vicilin from F₂ seeds segregated in a 1:2:1 parent A: additive: parent B ratio on gel electrophoresis, indicating that vicilin band patterns were controlled by pairs of co-dominant genes at single loci. The loci were not identified with the one demonstrated by Hynes (1968), nor was the relationship between the putatively different vicilin loci explored, although they were claimed to correlate with vicilin structural genes on the pea genome. Of the three loci identified, one (“Vca”) was subsequently identified with a related storage protein, convicilin (Matta and Gatehouse, 1982) while the others were identified with component polypeptides of vicilin of Mr < 35,000. None of the loci were mapped.

Elucidation of the synthesis of vicilin has shown that the initial products of the vicilin structural genes are 50,000 Mr precursors, of differing amino acid sequences, some of which are proteolytically cleaved after translation is complete to yield the “subunits” or component polypeptides of Mr < 35,000 observed in the protein as isolated (Gatehouse *et al.*, 1981, 1982). Variation in the 50,000 Mr subunits of vicilin is thus most likely to reflect variation in the structural genes for the protein rather than to be a result of post-translational modification (which may not be simply related to protein structure). However, variation in vicilin 50,000 Mr subunit band

patterns on SDS-PAGE has not been reported. In contrast, the well-established genetic variation in the α -subunits of legumin on SDS-PAGE (Casey, 1979) has led to the mapping of a legumin locus, *Lg-1*, to chromosome 7 in the pea genome, near the *r-tl* segment, where *r* (sometimes called *ra*) is the locus determining round (*R*) or wrinkled (*r*) seeds, and *tl* is the locus determining the presence of tendrils (*Tl*) or leaflets (*tl*) at the ends of the side-branches (*r*...5...*tl*...17...*Lg-1*) (Davies, 1980); Matta and Gatehouse, 1982). The latter authors also reported the mapping of the genetic locus for the storage protein related to vicilin, convicilin (*Cvc*), to a segment of chromosome 2 on the pea genome (*s*...18...*Cvc*...3...*k*) where *s* is the locus determining "sticky" (*s*) or "non-sticky" (*S*) seeds ("sticky" seeds adhere together in the pod), and *k* is the locus determining normal (*K*) or keel-like (*k*) wings on the flowers. The pea genome (4.8×10^9 bp) contains 7 chromosomes which range from 60–250 cross-over units in length (Blixt, 1977); the above data shows that the storage protein loci mapped are independent.

The present communication reports the mapping of a putative vicilin structural gene locus (*Vc-1*) based on genetic variation in vicilin 50,000 Mr subunit band patterns on SDS-PAGE.

KEY TO GENE SYMBOLS (From *The Pisum-Genebank Gene-Symbols*, Blixt, S., Weibullsholm Plant Breeding Institute, Landskrona, Sweden (1981))

<i>a</i> ;	absence of anthocyanin pigments (white flowers)
<i>Br</i> ;	presence of bracts on flowers
<i>bt</i> ;	pod apex pointed
<i>Cvc</i> ;	convicilin SDS-PAGE band position
<i>D</i> ;	presence of axil colour
<i>dt</i> ;	short peduncle to first flower
<i>F, Fs</i> ;	seed testa with sharp, small violet spots
<i>fl</i> ;	absence of flecking (air pockets) on leaflets and stipules
<i>i</i> ;	seed cotyledons green (I: yellow)
<i>Kp</i> ;	keel coloured with anthocyanin
<i>le</i> ;	short internodes (dwarf habit)
<i>Lg-1</i> ;	legumin major α -subunits band pattern on SDS-PAGE
<i>M</i> ;	brown marbling of seed testa
<i>mifo</i> ;	close-set small and shallow impressions on testa
<i>Pl</i> ;	black hilum colour on seeds
<i>r</i> ;	wrinkled seed character (<i>R</i> ; round)
<i>td</i> ;	no dentation on leaflets
<i>Tra</i> ;	tragacanth on inside of testa (oily spot)
<i>Vc-1</i> ;	vicilin 50,000 Mr polypeptide band pattern on SDS-PAGE
<i>wsp</i> ;	waxless except upper surface of leaflets

Gene symbols with first letter capital indicate phenotypes dominant or codominant over the normal phenotype; others recessive

2. MATERIALS AND METHODS

Seeds of pea lines from the Weibullsholm collection (identified by number) were supplied by Dr S. Blixt, Landskrona, Sweden as were F_2 seeds from a cross of lines 360 and 611. In addition, seeds of a number of isogenic pairs of pea lines differing only at the *r* locus within each pair were supplied by Dr A. E. Slinkard. Reciprocal crosses between pea lines 1263 and 3080 were made, and the F_1 and F_2 progeny grown under greenhouse conditions at the Botanic Garden, Durham.

PLATE 1. SDS-polyacrylamide gel electrophoresis (12 per cent acrylamide gel) of total protein extracts of seeds of different lines of peas (numbered), (400 μ g meal/track) and of vicilin from line Feltham First (Vc) and from lines 360 and 611 (360 Vc, 611 Vc? (20 μ g protein/track). Variation in vicilin 50,000 M (Vc-1) and legumin α -subunit (Lg-1) band patterns is indicated.

PLATE 2. SDS-polyacrylamide gel electrophoresis of total protein extracts of seeds of lines 1263 and 3080, and F₁ and F₂ seeds from their cross. Conditions as in plate 1. Vc-1 indicates variation in vicilin 50,000 Mr subunit band pattern, Lg-1 variation in legumin α-subunit band pattern.

PLATE 3. SDS-polyacrylamide gel electrophoresis (17 per cent acrylamide gel) of total protein extracts of seeds from isogenic round/wrinkled pea lines (T), compared with corresponding "Western" blots of vicilin subunits (Vc). R indicates round seed phenotype, r wrinkled seed phenotype. Other conditions as in plate 1.

Total protein extracts of single or half mature pea seeds were made and analysed by SDS-polyacrylamide gel electrophoresis on 12 per cent acrylamide gels as previously described (Matta *et al.*, 1981; Laemmli, 1970). Vicilin subunit bands were transferred from SDS polyacrylamide gels to nitrocellulose paper by electro-blotting, and were subsequently detected using affinity-purified antivvicilin IgG, goat anti-rabbit IgG (peroxidase conjugated) and staining with 4-chloro-1-naphthol according to standard protocols (Towbin *et al.*, 1979).

Chi-square values were calculated according to Fisher (1925). The F_2 segregation data were used to give crossing over values by the "product ratio" method of Immer (1930).

3. RESULTS

(i) Variation in vicilin subunit band patterns

Plate 1 shows the total protein subunit band patterns of different pea lines. In general, little variation in the band patterns of vicilin subunits at approx. 50,000 Mr or 33,000 Mr was observed, in contrast to the pronounced variation in legumin α subunits (4 different patterns in 8 lines tested) and frequent, although less pronounced, variations in the convicilin band (3 different patterns in 8 lines tested). However, line 3080 showed a double band at 50,000 Mr whereas all other lines showed a single thicker band; the latter pattern was also given by a further 8 lines screened. Line 611 gave a double banded pattern similar to 3080. No variation in the vicilin 33,000 Mr "subunits" was observed in this study, and variations in other vicilin polypeptides were not examined. A cross of lines 3080 and 1263 was selected as suitable for testing the segregation between the variation in vicilin band pattern and that in legumin α subunits; in addition, the seed surface round/wrinkled gene *r* differed between these lines (Genotypes: 1263 = *a*, *Br*, *dt*, *Tra*; 3080 = *a*, *r*, *le*). F_2 seeds from a cross of lines 360 and 611 were also examined (Genotypes 360 = *a*, *wsp*, *fl*, *bt*, *mifo*, *Tra*, *i*, *r*; 611 = *Kp*, *td*, *D*, *Pl*, *M*, *F*, *Fs*).

(ii) Inheritance of vicilin 50,000 Mr subunit

Ten F_1 seeds from each reciprocal cross of lines 1263 and 3080 showed additive inheritance patterns for the vicilin 50,000 Mr subunit band pattern; the F_1 patterns were the same irrespective of which parent was the male parent and no segregation was observed (see plate 2). These results indicate that there is no maternal effect in the inheritance of the major vicilin subunit and the parental lines were homozygous. 308 F_2 seeds were examined, and showed the expected monogenic inheritance and dominance of the *r* gene (table 1). The segregation of the band patterns for the α subunits of legumin and the vicilin 50,000 Mr subunits in the F_2 seeds is shown in plate 2. The observed frequencies of the parental and hybrid band patterns of the legumin α - and vicilin 50,000 Mr subunits are given in table 1, and support the hypothesis that the vicilin 50,000 Mr subunit band pattern is controlled by a single pair of co-dominant alleles, like that (*Lg-1*) controlling the legumin α -subunit band pattern. This locus is subsequently referred to as *Vc-1*. That the observed variation in band patterns was actually due to vicilin polypeptides was confirmed by transfer of the protein subunits from an SDS-PAGE

TABLE 1

Observed frequency, χ^2 values and probability range of the characters studied in the F_2 progeny of the cross "1263 $\times$ 3080"

Character	F_2 classes and observed frequency			χ^2
Vicilin 50,000 Mr subunit (Vc-1)	Vc ⁽¹²⁶³⁾ 68	Vc ^(F₁) 160	Vc-1 ⁽³⁰⁸⁰⁾ 80	1.40 ^{ns}
Legumin α subunit (Lg-1)	Lg-1 ⁽¹²⁶³⁾ 84	Lg-1 ^(F₁) 158	Lg-1 ⁽³⁰⁸⁰⁾ 66	2.31 ^{ns}
Seed surface (R/r)	R ⁽¹²⁶³⁾ 238	r ⁽³⁰⁸⁰⁾ 70	—	0.84 ^{ns}

ns = Not significant ($p > 0.3$).

R = Seed surface (Round) line 1263.

r = Seed surface (wrinkled) line 3080.

analysis of total extracts of parental, F_1 and F_2 seeds to nitrocellulose and specific detection of vicilin polypeptides by antivicalin IgG ("Western" blotting). A strong reaction was observed with the variant 50,000 Mr subunits (data not presented). Other vicilin polypeptides which reacted with the antivicalin IgG were invariant.

(iii) Mapping the vicilin gene locus

In order to establish whether or not the genetic locus for the vicilin 50,000 Mr subunit band pattern was linked to the markers r and Lg-1, the F_2 data were used to calculate crossover values (table 2). No recombinants were observed between the Vc-1 pattern of line 1263 and wrinkled seed surface (r) leading to a crossover value of 0 between the Vc-1 and r loci, and suggesting that these genes are closely linked to the extent of being within less than 2 crossover units of each other. In confirmation of earlier results, the major legumin gene Lg-1 was found to be linked to gene r on chromosome 7 (crossover value 36.2 ± 4.9). A crossover value of 29.5 ± 3.3 between Lg-1 and Vc-1 was also determined; in this case the genes are in coupling phase (table 2) so this figure is likely to be more accurate. No linkage to other genes segregating in this cross was observed. Examination of 181 F_2 seeds from the cross of lines 360 and 611 also showed no recombinants between the wrinkled seed surface r phenotype and line 611 (Vc-1) pattern, and gave a crossover value of 16.2 ± 3.1 per cent between Lg-1 and Vc-1 (coupling phase), in reasonable agreement with data from the other cross. Although these lines are known to contain transpositions involving chromosome 7 (Blixt, 1977) it is most unlikely that this would affect the very close linkage between Vc-1 and r .

The close linkage of the vicilin locus Vc-1 with the r locus was further supported by examination of seeds from pairs of isogenic lines differing at the r locus. SDS-polyacrylamide gel electrophoresis of total protein extracts of these seeds showed that within each pair band patterns were identical, except that in 3 out of the 5 isogenic pairs examined one member of the

TABLE 2
F₂ classes, observed frequency, χ^2 and crossover values and standard error of the pair of characters studied in the cross "1263 $\times$ 3080"

Character pair	<i>F₂</i> classes and observed frequency				χ^2	Crossover value $\pm$ probable error
Legumin type; Seed surface Lg-1; R/r (Repulsion)	Lg-1 ⁽³⁰⁸⁰⁾ R 164	Lg-1 ⁽¹²⁶³⁾ R 74	Lg-1 ⁽³⁰⁸⁰⁾ r 60	Lg-1 ⁽¹²⁶³⁾ r 10	9.6*	36.2 $\pm$ 4.9
Vicilin type; Seed surface Vc-1; R/r (Repulsion)	Vc-1 ⁽³⁰⁸⁰⁾ R 170	Vc-1 ⁽¹²⁶³⁾ R 68	Vc-1 ⁽³⁰⁸⁰⁾ r 70	Vc-1 ⁽¹²⁶³⁾ r 0	23.7**	0
Vicilin type; Legumin type Vc-1; Lg-1 (Coupling)	Vc-1 ⁽³⁰⁸⁰⁾ Lg-1 ⁽³⁰⁸⁰⁾ 193	Vc-1 ⁽¹²⁶³⁾ Lg-1 ⁽³⁰⁸⁰⁾ 47	Vc-1 ⁽³⁰⁸⁰⁾ Lg-1 ⁽¹²⁶³⁾ 31	Vc-1 ⁽¹²⁶³⁾ Lg-1 ⁽¹²⁶³⁾ 37	33.0**	29.5 $\pm$ 3.3

* Significant ($p > 0.02$).

** Highly significant ($p > 0.01$).

R = Seed surface (Round) of line 1263.

r = Seed surface (wrinkled) of line 3080.

Line 3080 was taken as dominant for Vc-1 and Lg-1 band patterns; thus (3080) includes seeds showing *F₁* protein patterns.

pair had an extra band in the vicilin 50,000 Mr region, at approx. 48,000 Mr (plate 3). This band reacted with antivicalin IgG after transfer to nitrocellulose using a "Western" blotting procedure. Since these seeds were F_8 selections, *Vc-1* and *r* must be closely linked for the different phenotypes at the two loci to have segregated together. Although an exact figure cannot be calculated from the small sample analysed, *Vc-1* and *r* are suggested to be within 10 crossover units of each other, since this distance implies co-segregation in approx. 50 per cent of F_8 selections, assuming the parental lines differed in both their *Vc-1* and *r* phenotypes.

4. DISCUSSION

The locus identified in the present study is likely to represent a structural gene for vicilin for the reasons discussed above, *i.e.*, that the observed difference in 50,000 Mr subunit band patterns corresponds most nearly of all vicilin polypeptides to the initial translation products, and thus to genomic coding sequences. It is possible, however, that the differences in 50,000 Mr subunit band patterns observed on SDS-PAGE are the result of some kind of post-translational modification not determined by structural genes, *e.g.* differences in carbohydrate side chains, but this is unlikely since the carbohydrate moiety of vicilin is known to be associated almost exclusively with the 16,000 Mr polypeptide (Davey *et al.*, 1981). This locus relates to a polypeptide different from those associated with the loci identified by Thomson and Schroeder (1978), although because of the precursor-product relationships of vicilin polypeptides of approx. 50,000 Mr and less than 35,000 Mr respectively, it is not necessarily different from their loci "*Vcb*" or "*Vcc*". The symbol *Vc-1* is used in accordance with the recommendations of the Pisum Genetics Association (Blixt, 1977) to allow for further loci, if shown to be distinct when mapped, to be designated *Vc-2*, etc.

Further vicilin loci are possible, since vicilin is known to be encoded by a small family (< 10 copies) of genes on the pea chromosome (Gatehouse *et al.*, 1983; Lycett *et al.*, 1983). Legumin subunits, which are also encoded by a small gene family (3-4 members; Croy *et al.*, 1982) map to more than one locus, (Casey, 1979; Matta and Gatehouse, 1982). However, some or all of the vicilin structural genes may be arranged in one or more closely linked arrays which would result in the number of "classical" vicilin loci being considerably fewer than the number of genes determined by DNA hybridisation. The present vicilin locus is not closely associated with either legumin or convilinin loci and thus vicilin gene expression must be controlled independently of the other storage proteins.

Close linkage of the vicilin locus with the *r* locus is interesting in view of reports that the legumin: vicilin ratio in peas is affected by the round/wrinkled genotype (Davies, 1980; Schroeder, 1982), although the actual amounts of legumin in mg per seed does not differ significantly between round and wrinkled lines (Schroeder, 1982). It is thus perhaps tempting to postulate a direct effect of the *r* locus genotype on vicilin synthesis, as was suggested for legumin synthesis (Davies, 1980); however, even if this were the case, the pleiotropic effects of the wrinkled phenotype (Shia and Slinkard, 1977) would make the existence of such a direct control very difficult to demonstrate.

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