Inheritance of Viral Bean Leaf Roll Tolerance in Peas

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Abstract. The inheritance of tolerance to infection by bean leaf roll luteovirus (BLRV) in Pisum sativum L. was studied in the cross of cv. Parlay (sensitive to BLRV infection) × cv. Oregon Sugarpod II (BLRV tolerant). The parents, reciprocal F1, back-crosses, F2, and 234 random F3 families were screened in 1986 and 1987 in the field at Twin Falls, Idaho, under natural BLRV inoculation by aphids. Overall disease index scores for the F1, F2, and F3 were about intermediate between indices of the parents, with the F1 usually slightly higher than midparent values. Backcross disease indices were intermediate between the F1 and the respective parent involved. Distribution of individual F3 family indices was continuous and semi-normal. BLRV-sensitivity ranges within parents and selected cultivars, as well as segregating populations showed continuous variation and differed between the 2 years, suggesting that expression of a major gene was significantly influenced by natural variation in BLRV inoculation pressure and timing. An apparent “additive gene action” was probably an artifact of nonuniform timing and levels of infection within plant populations. Chi-square analyses of segregating populations indicated that a major recessive gene, called lrv, conferred BLR disease tolerance.

The “yellows disease” of pea caused by BLRV was first reported by Boning (1927), though its etiology was unknown. Quantz and Volk (1954) established that the “Blattroll” (leaf roll) disease in fababean (Vicia faba L.) was caused by a persistently aphid-transmissible virus, and Hubbeling (1956) recognized that this virus also caused “top yellows” of peas in The Netherlands. Since that time, the virus has been called “pea top yellows virus” (Crampton, 1966), “Jaunisse du pois” (pea pillly et al., 1977), “legume yellows virus” (Duffus, 1979), and “pea leaf roll virus” (Hampton, 1983). The present term, BLRV (Ashby, 1984; Waterhouse et al., 1988) refers to the fababean host crop in which the virus was originally described.

Concerted efforts to evaluate BLRV-resistance in peas were begin by Hubbeling (1956) who rated some 273 cultivars and lines naturally inoculated in field plots of the central and southern districts of The Netherlands, where “top yellows” was endemic and very destructive to peas. Preliminary evidence suggested that BLRV-resistance was conferred by “one or more dominant genes”.

Results of pea luteovirus resistance studies with peas were reported simultaneously by Drijfhout (1968) and Crampton and Watts (1968). Results reported by Wilson and Close (1973), Grylls and Peak (1969), and Johnstone and Duffus (1984) suggest that Crampton and Watts probably described resistance to either (or both) beet western yellows or subterranean clover redleaf luteoviruses. Data obtained from glasshouse inoculation techniques by Drijfhout indicated that BLRV resistance was attributable to a single recessive gene, designated lr, although variations in segregation ratios were noted. From segregation ratios of field-inoculated breeding progenies, Crampton and Watts (1968) concluded that resistance to “pea leaf-roll virus” (probably luteovirus complex) infection “was controlled by an additive system of inheritance, with moderate resistance being dominant to both susceptibility and high resistance”.

BLRV was not recognized as a major pea pathogen in the Pacific Northwestern United States until 1980 (Hampton, 1983), when crops of most susceptible pea cultivars grown in southern Idaho were destroyed by this virus. As had been true for many decades in The Netherlands (Hubbeling, 1956), alfalfa was the principal inoculum reservoir for BLRV and also the overwintering host for the vector, the pea aphid (Acrithosiphon pisum Harris). Since 1980, endemic recurrence of BLRV has enabled seed company research personnel in southern Idaho, starting largely with their own breeding lines, to make significant advances in developing superior new BLRV-resistant cultivars. This paper reports responses of F1 progenies and segregation ratios of F2 populations and F3 families from crosses of BLRV-tolerant and -sensitive parents exposed to consecutive natural BLRV-inoculation in southern Idaho.

Materials and Methods

No examples of Pisum immunity to BLRV were known to us when we began this study. Therefore, selection of parents was based on reactions of cultivars and breeding lines to natural inoculations with BLRV during 1980–83 at Twin Falls, Idaho. The edible-podded, pea enation mosaic virus-tolerant ‘Oregon Sugarpod II’ (OSP; Baggett, 1982) was representative of numerous BLRV-tolerant cultivars (i.e., susceptible to infection, but producing nearly normal plants and yields when diseased) and was selected as the BLRV-tolerant parent (Pt). The commercial canning cultivar Parlay was selected as the BLRV-sensitive parent (Ps) (severely stunted, chlorotic, and nonfruitful when infected). Reciprocal crosses and backcrosses were made

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Abbreviations: BLRV, bean leaf roll luteovirus.
in the greenhouse using the standard method of emasculation and pollination of unopened flowers. F1 and F2 seeds were also produced in the greenhouse. F1 families were derived as seed from random individual F1 plants.

Reactions of parents and progenies to BLRV inoculation were tested under field conditions at the breeding grounds of Rogers Brothers Seed Co., Twin Falls. In 1986, 100 F1 families (50 of each reciprocal), parents, F1, and backcrosses of ‘Parlay’×OSP were planted on 7 Apr. adjacent to an established planting of alfalfa. Generally, 15 to 20 seeds were planted for each family. Parent, F1, and backcross seeds were planted in two to four replications each, randomly distributed among the plots of F1 families. In the 1987 test, planted 21 Apr., the arrangement was similar to that of 1986, except that in addition to 134 F1 families, F2 populations were available and were divided into three to four randomly distributed plots of 55 to 60 plants for each reciprocal. Several proprietary pea cultivars were planted adjacent to the genetic populations and were also scored for severity of bean leaf roll (BLR) symptoms.

At the peak of symptom expression, 90 days after planting in 1986 and 62 days after planting in 1987, individual plants were scored by means of symptom severity classes: 1 = no observable reduction in growth or other damage, slight yellowing sometimes visible; 2 = slight stunting with typical symptoms: general yellowing, sometimes with marginal and/or interveinal chlorosis of lower or terminal leaves; 3 = moderate stunting and chlorosis, plants still produce pods; 4 = severe stunting or death, no pods produced. A disease severity index (DI) was calculated for each plot by the formula:

\[
DI = \left[ (No. \text{ plants} \times \text{score} \ 1) + (No. \times 2) + (No. \times 3) + (No. \times 4) \right] \times 100 / \text{Total no. of plants}
\]

providing a range of indices from 100 to 400.

Data from reciprocal crosses were considered equivalent and combined for analysis and presentation. Selected plants were tested for presence of BLRV by enzyme-linked immunosorbent assays (ELISA), using BLRV-specific immunoglobulin G produced in the laboratory of R.O.H.

Results

Within-line ranges of symptom intensities. Bean leaf roll was ideally severe for inheritance studies in the field in both 1986 and 1987, causing extensive damage to BLRV-sensitive controls, with greater damage in 1987 than in 1986. Even under this inoculum pressure, however, plants of tolerant and sensitive parents and of all segregating progenies exhibited the full range of symptom intensities (Table 1), from symptomless (class 1) to severe yellows, stunting, or plant death (class 4). For instance, some individual plants of OSP were assigned infection scores of 4.0 and disease indices of some plots exceeded levels expected for tolerant genotypes. Yet at the same time, some plants of the BLRV-sensitive parent ‘Parlay’ expressed no symptoms. Some symptomless plants could have escaped BLRV inoculation; however, assays of BLRV-tolerant and -sensitive genotypes by ELISA indicated that most symptomless plants were BLRV-infected.

Despite an excess of symptomless (“tolerant”) F1 and BC plants in 1986, data from both years suggest that a major single recessive gene confers tolerance to the BLR disease.

Recessiveness of BLR disease tolerance. The F1 plants also exhibited the entire range of BLR symptoms. In 1986 (lesser BLRV inoculation pressure), the distribution of F1 plants in

| generation | score class | no. of individuals | generation index |
|------------|-------------|-------------------|-----------------|
| 1986       |             |                   |                 |
| Pt         | 74          | 3                 | 2               | 4                 | 79 | 109 |
| Ps         | 8           | 5                 | 22              | 28               | 63 | 311 |
| F1         | 31          | 16                | 15              | 9                | 71 | 203 |
| F2         | 495         | 391               | 379             | 211              | 1476 | 221 |
| BCt        | 74          | 35                | 22              | 6                | 137 | 171 |
| BCs        | 19          | 32                | 53              | 27               | 131 | 267 |
| 1987       |             |                   |                 |
| Pt         | 125         | 42                | 11              | 6                | 184 | 145 |
| Ps         | 3           | 13                | 41              | 114              | 171 | 356 |
| F1         | 6           | 8                 | 8               | 13               | 35  | 280 |
| F2         | 91          | 57                | 76              | 125              | 349 | 267 |
| BCt        | 824         | 402               | 704             | 1003             | 2933 | 264 |
| BCs        | 71          | 35                | 28              | 65               | 199 | 244 |
|            | 11          | 25                | 65              | 97               | 198 | 325 |

\begin{table}
\begin{small}
\begin{tabular}{lcccc}
\hline
\textbf{generation} & \textbf{score class} & \textbf{no. of individuals} & \textbf{generation index} \\
\hline
\textbf{1986} & & & \\
Pt & 74 & 3 & 2 & 4 & 79 & 109 \\
Ps & 8 & 5 & 22 & 28 & 63 & 311 \\
F1 & 31 & 16 & 15 & 9 & 71 & 203 \\
F2 & 495 & 391 & 379 & 211 & 1476 & 221 \\
BCt & 74 & 35 & 22 & 6 & 137 & 171 \\
BCs & 19 & 32 & 53 & 27 & 131 & 267 \\
\textbf{1987} & & & \\
Pt & 125 & 42 & 11 & 6 & 184 & 145 \\
Ps & 3 & 13 & 41 & 114 & 171 & 356 \\
F1 & 6 & 8 & 8 & 13 & 35 & 280 \\
F2 & 91 & 57 & 76 & 125 & 349 & 267 \\
BCt & 824 & 402 & 704 & 1003 & 2933 & 264 \\
BCs & 71 & 35 & 28 & 65 & 199 & 244 \\
\hline
\end{tabular}
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\caption{Frequency distribution of individual plant bean leaf roll infection scores in ‘Parlay’×Oregon Sugarpod II’ generations.}
\end{table}

\footnote{Pt = tolerant parent, Ps = sensitive parent, BCt = backcross F1 tolerant parent, BCs = backcross F1 × sensitive parent.}

\footnote{Overall population index calculated from individual plant scores (range 100 to 400). This value was identical or nearly identical to means of plot indices for the same population.}

score classes 1-4 was skewed toward tolerance, with 56% developing BLR symptoms. In 1987 (greater BLRV inoculation pressure), the distribution was skewed toward sensitivity, with 85% developing BLR symptoms, suggesting recessiveness of tolerance. The overall DI for F1 plants approximated midparent values in 1986 (F1 DI = 203 vs. MP DI = 210) and exceeded midparent values in 1978 (F1 DI = 280 vs. MP DI = 250). Likewise, disease indices for backcross populations were intermediate between the values for F1 and the respective backcross parent. Most lines of evidence, therefore, suggest the expression of a major single recessive gene under the influence of complementary genes and variable inoculation pressures.

For instance, the sum of F1 plants (1987) classified under BLR-severity scores 2 + 3 + 4 was about three times that for plants classified under severity score 1. The data for segregating populations were therefore reorganized into columns, respectively, for sensitive and tolerant progenies for comparison with the severity scores for parents (Table 2). The single-recessive-gene premise for BLRV tolerance was also tested for the five segregating populations by $\chi^2$ analysis (Table 2). The ratio of tolerant : sensitive plants in the 1986 BCt and 1987 F2 populations closely fit ratios of 1:1 and 1:3, respectively, as expected for the expression of a single recessive gene. Differences between observed and expected ratios of tolerant : sensitive plants were significant in the other three segregating populations. In each case, the lack of fit was due to a deficiency of tolerant plants, probably attributable to our inability to properly classify “symptomless” and “slightly BLRV-affected” plants. Nevertheless, the observed segregation in even these three populations suggested the expression of a single recessive gene. We propose the symbol $lr$ for the gene enabling genotypes to yield well even when infected with BLRV.

The frequency distribution of F1 family disease indices shows the lower number of plants with severe BLR in 1986 (Fig. 1A), than 1987 (Fig. 1B), reflecting the greater BLRV inoculation pressure of 1987. Accompanying the greater inoculum pressure of 1987 was a clearer distinction between tolerant and sensitive
Table 2. Fitness test for the ratio of tolerant (T, score class 1): sensitive (S, score classes 2-4) plants of five segregating populations, against hypothetical monogenic ratios.

| Generation | Observed | Expected | Ratio | $\chi^2$ | $P$ |
|------------|----------|----------|-------|---------|-----|
|            | T         | S        |       |         |     |
| 1986       | T         | S        |       |         |     |
| Pt         | 74        | 5        | 79.0  | 0       |     |
| Ps         | 8         | 55       | 0.0   | 63      |     |
| F₁         | 31        | 40       | 0.0   | 71      |     |
| F₂         | 495       | 981      | 553.5 | 922.5   | 3:5 | 9.9 | <0.01 |
| BCt        | 74        | 63       | 68.5  | 68.5    | 1:1 | 0.9 | 0.50-0.20 |
| BCs        | 19        | 112      | 11.2  | 112     | 1:10 | 0.1 | 0.9     |
| 1987       | T         | S        |       |         |     |
| Pt         | 125       | 59       | 184.0 | 0.0     |     |
| Ps         | 3         | 168      | 0.0   | 171.0   |     |
| F₁         | 6         | 29       | 0.0   | 35.0    |     |
| F₂         | 91        | 258      | 87.2  | 261.8   | 1:3 | 0.2 | 0.95-0.50 |
| F₃         | 824       | 2109     | 1099.9| 1833.1  | 3:5 | 110.7 | <0.01 |
| BCt        | 71        | 128      | 99.5  | 99.5    | 1:1 | 16.3 | <0.01 |
| BCs        | 11        | 187      | 0.0   | 198.0   |     |

Pt = tolerant parent ('Oregon Sugarpod II'); Ps = sensitive parent ('Parlay'), BCt = backcross $F₁$ x tolerant parent, BCs = backcross $F₁$ x sensitive parent.

Fig. 1. Frequency distribution of BLRV disease indices, $F₃$ families of 'Parlay' x 'Oregon Sugarpod II'; (A) 1986, (B) 1987.

Sensitivity of cultivars to BLRV. The range of BLRV disease indices among proprietary pea cultivars included in progeny-screening plots is presented in Table 3. Plants of all cultivars exhibited the complete range of BLRV-severity scores, from symptomless to severe yellow, stunting, and plant death. Some were more sensitive to BLRV than 'Parlay' and others more tolerant than OSP. Extensive unpublished trial results from 1980 to 1987, provided by seed company research personnel, corroborate our observed BLRV-reaction diversity typical for all known commercial cultivars and breeding lines.

Table 3. Responses of selected commercial pea cultivars to natural BLRV inoculation, Twin Falls, Idaho, 1987.

| Cultivar | Disease index | Cultivar | Disease index |
|----------|---------------|----------|---------------|
| 1 Oregon 523 | 109          | 13       | 278           |
| 2         | 123           | 14       | 326           |
| 3         | 135           | 15       | 331           |
| 4 OSP²    | 147           | 16       | 333           |
| 5         | 163           | 17       | 340           |
| 6         | 170           | 18       | 356           |
| 7 Progress 9 | 188         | 19       | 364           |
| 8         | 190           | 20       | 370           |
| 9         | 223           | 21       | 388           |
| 10        | 245           | 22       | 390           |
| 11        | 249           | 23       | 400           |
| 12        | 264           |          |               |

*Mean disease indices were obtained from two or more plot replications. The full range of BLR severity types was exhibited among the plants of most cultivars. The cultivar names of numbered cultivars were omitted at the request of seed companies.

Discussion

Monogenic inheritance of BLRV tolerance. The report of Drijfhout (1968) that resistance was a monogenic recessive character differed from the conclusion of Hubbeling (1956) that resistance was conditioned by one or more dominant genes. Each of these studies was apparently based on a completely qualitative classification with no provision for variations in symptom severity. By accounting for number of infected plants and degree of BLR severity per segregating population, we were able to observe the operation of a single recessive gene, $lrv$, under the influence of extrinsic factors and to examine possible interactions with other genes. Extrinsic factors would have included yearly variability in BLRV inoculation pressure (most apparent in Table 1), probable variability in time of plant infection relative to stage of plant growth, and probable plant-to-plant microclimatic effects on symptom development. Interaction of $lrv$ with undefined minor genes may have caused deviations from expected ratios, based on the action of a single recessive gene. Such minor gene effects might have been inferred from 1986 data, when more plants escaped infection, as compared with 1987 (Table 1). Under the BLRV inoculum pressure of 1987, however, data configurations suggesting minor-gene effects almost disappeared, e.g., the generation index for BC, approximated that of P, and the generation index for BC approximated a value intermediate between the two parents.

Responses within and among commercial pea cultivars to BLRV inoculation (Table 3) suggest two separate influences. Intracultivar variability in BLRV disease indices (i.e., the full range of BLR severities, in most cases) suggests the influence of the previously mentioned extrinsic factors on disease development. However, the continuum of BLRV tolerance levels among cultivars suggests either separate additive tolerance-conferring genes or the action of undefined minor genes on the expression of gene $lrv$.

Possible multigenic corollaries. McKenzie et al. (1985) exposed random $F₅$ and $F₆$ lines from four crosses to natural or artificial inoculations with barley yellow dwarf luteovirus, observed essentially continuous distributions of resulting disease indices, and concluded that oat plant tolerance to barley yellow dwarf was conditioned by two to four quantitatively inherited genes. It is conceivable that Pisum genes other than $lrv$ could...
contribute to BLRV tolerance or resistance, and that Hubbeling (1956) and Drijfhout (1968) encountered genetic systems for tolerance/resistance that were distinct from each other.

Several commercial pea breeding lines developed recently in southern Idaho exhibit BLRV resistance or immunity greatly exceeding the highest levels of BLRV tolerance shown in Table 3 or in other known cultivars. These lines have remained symptomless and free from ELISA-detectable BLRV, even when adjacent sensitive cultivars were destroyed by BLRV infection. According to the originators of these lines, they were derived through “pyramiding” of several distinct BLRV-tolerant genotypes, rather than by crosses to singular sources of immunity. This privileged information suggests that multiple genes may contribute additively in conferring resistance against viral synthesis. Multiple parallel host genes typically have been revealed when viral pathotypes are tested against diverse plant genotypes (Drijfhout, 1978; Provvidenti and Alconero, 1988a, 1988b). To date, however, we have no evidence of pathotype diversity among BLRV isolates from southern Idaho. Examination of 11 BLRV isolates from that area in 1983–84 indicated that all performed comparably on several selected Pisum genotypes (R.O.H., unpublished). Conversely, the “legume yellows” isolate from California (Duffus, 1978) is clearly distinguishable as a separate BLRV pathotype (R.O.H. and J.E. Duffus, unpublished), and other pathotypes of BLRV likely exist.

In the absence of allelism tests, the relationship between the lrv gene of cv. OSP and the lr gene reported by Drijfhout cannot be known. We have proposed the term lrv, consistent with current convention (e.g., Provvidenti and Alconero, 1988a, 1988b), indicating tolerance to a viral pathogen.

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