J. AMER. SOC. HORT. SCI. 116(4):728-731. 1991.

Inheritance of Viral Bean Leaf Roll Tolerance in Peas

James R. Baggett
Department of Horticulture, Oregon State University, Corvallis, OR 97331

R.O. Hampton
U.S. Department of Agriculture, Agricultural Research Service, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331

Additional index words. vegetable breeding, *Pisum sativum*

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 F_1, back-crosses, F_2, and 234 random F_3 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 F_1, F_2, and F_3 were about intermediate between indices of the parents, with the F_1 usually slightly higher than midparent values. Backcross disease indices were intermediate between the F_1 and the respective parent involved. Distribution of individual F_3 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 yellows) (Roland, 1955), "Michigan alfalfa virus" (Thottappilly 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 *(Acyrthosiphon 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 F_1 progenies and segregation ratios of F_2 populations and F_3 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

Received for publication 9 Mar. 1990. Oregon Agricultural Experiment Station Technical Paper no. 9177. The assistance and cooperation of pea seed companies operating in the Twin Falls, Idaho, area are gratefully acknowledged. Special thanks are extended to John Morris, Rogers Brothers Seed Co., who arranged for use of company plot areas near plantings of alfalfa and for the planting and care of test plots and to Paul Moser, Rogers Brothers, who critically reviewed data in this manuscript. The cost of publishing this paper was defrayed in-mpart by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Abbreviations: BLRV, bean leaf roll luteovirus.

in the greenhouse using the standard method of emasculation and pollination of unopened flowers. F_2 and F_3 seeds were also produced in the greenhouse. F_3 familes were derived as seed from random individual F_2 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 F_3 families (50 of each reciprocal), parents, F_1, 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, F_1, and backcross seeds were planted in two to four replications each, randomly distributed among the plots of F_3 families. In the 1987 test, planted 21 Apr., the arrangement was similar to that of 1986, except that in addition to 134 F_3 families, F_2 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:

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

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") F_1 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 F_1 plants also exhibited the entire range of BLR symptoms. In 1986 (lesser BLRV inoculation pressure), the distribution of F_1 plants in 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 F_1 plants approximated midparent values in 1986 (F_1 DI = 203 vs. MP DI = 210) and exceeded midparent values in 1978 (F_1 DI = 280 vs. MP DI = 250). Likewise, disease indices for backcross populations were intermediate between the values for F_1 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.

Table 1. Frequency distribution of individual plant bean leaf roll infection scores in 'Parlay'× Oregon Sugarpod II' generations.

Generation[z]	No. of individuals: Score class 1	2	3	4	Total	Generation index[y]
			1986			
Pt	74	3	2	0	79	109
Ps	8	5	22	28	63	311
F_1	31	16	15	9	71	203
F_3	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
F_1	6	8	8	13	35	280
F_2	91	57	76	125	349	267
F_3	824	402	704	1003	2933	264
BCt	71	35	28	65	199	244
BCs	11	25	65	97	198	325

[z]Pt = tolerant parent, Ps = sensitive parent, BCt = backcross F_1 × tolerant parent, BCs = backcross F_1 × sensitive parent.
[y]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.

For instance, the sum of F_2 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 χ^2 analysis (Table 2). The ratio of tolerant : sensitive plants in the 1986 BCt and 1987 F_2 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 *lrv* for the gene enabling genotypes to yield well even when infected with BLRV.

The frequency distribution of F_3 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[z]	Observed T	Observed S	Expected T	Expected S	Ratio tested	χ^2	*P*
			1986				
Pt	74	5	79.0	0			
Ps	8	55	0.0	63			
F_1	31	40	0.0	71			
F_3	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	0.0	131.0			
			1987				
Pt	125	59	184.0	0.0			
Ps	3	168	0.0	171.0			
F_1	6	29	0.0	35.0			
F_2	91	258	87.2	261.8	1:3	0.2	0.95–0.50
F_3	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			

[z]Pt = tolerant parent ('Oregon Sugarpod II'), Ps = sensitive parent ('Parlay'), BCt = backcross F_1 x tolerant parent, BCs = backcross F_1 x sensitive parent.

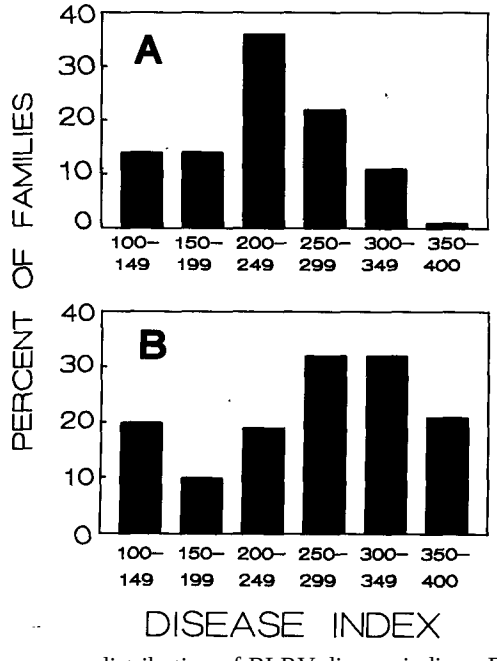

Fig. 1. Frequency distribution of BLRV disease indices, F_3 families of 'Parlay' × 'Oregon Sugarpod II'; **(A)** 1986, **(B)** 1987.

segregants, producing disease-index frequencies approximating a hi-modal distribution (Fig. 1B).

Sensitivity of cultivars to BLRV The range of BLR 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[z] 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	Perfection 326	326
3		135	15		331
4	OSP[y]	147	16		333
5		163	17	Jade	340
6		170	18	Parlay	356
7	Progress 9	188	19	Dark Skin Perf.	364
8		190	20		370
9		223	21		388
10		245	22		390
11		249	23		400
12		264			

[z]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.
[y]'Oregon Sugarpod II'.

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_s approximated that of P_s and the generation index for BC_t 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 F_5 and F_6 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.

Literature Cited

Ashby, J.W. 1984. Bean leaf roll virus. Description of plant viruses, No. 286. Cmwlth. Mycol. Inst. /Assn. Applied Biol., Kew, Surrey, England.

Baggett, J.R. 1982. 'Oregon Sugarpod' II edible pod pea. HortScience 17:93-94.

Boning, K. 1927. Die Blattrollkrankheit der Akerbohne; Untersuchungen über die Ubertragbarkeit der Blattrollkrankheit. Die Mosaikkrankheit der Akerbohne *(Vicia faba).* Forsch. Pflkr. Berlin 4:43-110.

Crampton, M.J. 1966. Disease-resistant pea cultivars. N.Z. J. Agr. Res. 9:152-156.

Crampton, M.J. and L.E. Watts. 1968. Genetic studies of pea leaf-roll (top-yellows) virus resistance in *Pisum sativum.* N.Z. J. Agr. Res. 11:771-783.

Drijfhout, E. 1968. Testing for pea leaf roll virus and inheritance of resistance in peas. Euphytica 17:224-235.

Drijfhout, E. 1978. Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus, with implications of strain identification and breeding for resistance. Agr. Res. Rpt. 872. Agricultural Publishing and Documentation, Wageningen, The Netherlands.

Duffus, J.E. 1979. Legume yellows virus, a new persistent aphid-transmitted virus of legumes in California. Phytopathology 69:217-221.

Grylls, N.E. and J.W. Peak. 1969. A virus complex of subterranean clover. Austral. J. Agr. Res. 20:37-45.

Hampton, R.O. 1983. Pea leaf roll in northwestern U.S.A. pea seed production areas. Plant Dis. 67:1306-1310.

Hubbeling, N. 1956. Resistance to top yellows and fusarium wilt in peas. Euphytica 5:71-86.

Johnstone, G.R. and J.E. Duffus. 1984. Some luteovirus diseases in Tasmania caused by beet western yellows and subterranean clover red leaf viruses. Austral. J. Agr. Res. 35:821-830.

McKenzie, R.I.H., P.A. Burnett, C.C. Gill, A. Comeau, and P.D. Brown. 1985. Inheritance of tolerance to barley yellow dwarf virus in oats. Euphytica 34:681-687.

Provvidenti, R. and R. Alconero. 1988a. Inheritance of resistance to a lentil strain of pea seedborne mosaic virus in *Pisum sativum.* J. Hered. 79:45-47.

Provvidenti, R. and R. Alconero. 1988b. Inheritance of resistance to a third pathotype of pea seedborne mosaic virus in *Pisum sativum.* J. Hered. 79:76-77.

Quantz, L. and J. Volk. 1954. Die Blattrollkrankheit der Akerbohne and Erbse, eine neue Viruskrankheit bei Leguminosen. Nachrbl. Dtsh. Pflschdienst 6:177-182.

Roland, G. 1955. Note sur un virus attaquant les legumineuses. Parasitica 11:66-68.

Thottappilly, G., Y-C.J. Kao, and G.R. Hooper. 1977. Host range, symptomatology, and electron microscopy of a persistent, aphid-transmitted virus from alfalfa in Michigan. Phytopathology 67:1451-1459.

Waterhouse, P.M., F.E. Gildow, and G.R. Johnstone. 1988. Luteovirus group. Description of plant viruses, No. 339. Assn. Applied Biologists, Inst. of Hort. Res., Wellesbourne, Warwick, U.K.

Wilson, J. and Close, R.C. 1973. Subterranean clover red leaf virus and other legume viruses in Canterbury. N.Z. J. Agr. Res. 16:305-310.