Inheritance of Resistance to Pea Seed-borne Mosaic Virus in Lentils[1]

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ABSTRACT

The USDA collection of lentils (*Lens culinaris* Medic.) was screened for resistance to pea seed-borne mosaic virus (PSbMV); PI lines 212610, 251786, 297745, and 368648 were found to be immune. After inoculation and an incubation period the virus was not recoverable by local lesion assay from the resistant lines, nor did the resistant lines develop symptoms or transmit the virus through seeds from inoculated plants. Two immune lines were used to study the inheritance of resistance. The F_2 progenies of crosses between PI 368648 and the susceptible cultivars 'Tekoa' and 'Precoz' segregated in a 3:1 ratio of susceptible to resistant plants, indicating that resistance was conferred by a single recessive gene. All F_2 progenies of a cross between PI 368648 and PI 212610 were 100% resistant, which indicated that the same recessive gene was present in the two sources. We propose that the gene symbol *sbv* be used to designate resistance to PSbMV in lentils. The PSbMV-infected plants could be identified under a range of temperatures. However, symptom expression was more severe at 24 and 30 C than at 18 C. Symptoms at all temperatures included stunting, leaf curling, and leaf size reduction. Vein clearing, tip bending, and tip necrosis occurred at 24 and 30 C, whereas, premature plant death only occurred at 30 C.

Additional index words: Host-plant resistance, *Lens culinaris* Medic, Temperature effects.

PEA seed-borne mosaic virus (PSbMV) is a potentially serious problem in peas (*Pisum sativum* L.) and lentils (*Lens culinaris* Medic.) wherever these crops are grown. The virus is a mosaic type transmissible (manually or by aphids) to all known pea and lentil cultivars (1, 4). The virus is also readily seed transmitted (5, 6). The frequency of seed transmission in lentils was estimated to range from 32 to 44% (5). Under field conditions the virus can overwinter in hairy vetch (*Vicia villosa* Roth) and volunteer peas. The virus is transmissible to susceptible pea cultivars planted nearby (8) and yield and seed quality are adversely affected (7). Virus-free seedstocks are used to control the virus. In addition, work is underway to develop virus-immune cultivars through the use of the *sbm* gene (2).

Pisum germplasm response to PSbMV infection is diverse and includes a barely visible mottle, slight to severe stunting, leaf curling, vein clearing, rosetting, and prolonged flowering (3, 4, 5). Distorted flowers may develop and most fail to produce pods. The pods that are produced tend to be small and distorted; they contain misshapen seeds, often with split seedcoats (6).

Increasing the incubation temperature from 16 to 28 C accelerated virus symptom expression in peas (9). However, the overall and final severity of symptoms was unaffected by temperature. Disease expression in response to varied temperatures is unknown in susceptible lentil cultivars.

Symptoms of PSbMV in lentils are similar to those in peas and consist of plant stunting, leaf rolling, reduction in leaf size, stem twisting, distorted flowers, and reduced seed set (5). Because of the high incidence of seed transmission and the susceptibility of commercial lentil cultivars, the 40,500-ha lentil crop in the Pacific Northwest is vulnerable to PSbMV. Control of the disease at present depends on the use of virus-free seedstocks, whereas the ultimate means of control would be cultivars resistant to PSbMV.

The identification of PSbMV-resistant lentil germplasm, the inheritance of resistance, and the influence of temperature on disease expression is reported here.

MATERIALS AND METHODS

Screening The PI Collection. One plant from each of 568 lentil lines obtained from the USDA Plant Introduction Station, Pullman, Wash. was used in the initial greenhouse screening test. When the seedlings had three sets of compound leaves, they were dusted with 400-mesh silicon carbide (carborundum powder) and mechanically inoculated by finger rubbing with crude juice prepared by grinding 0.5 g of PSbMV-infected pea leaf tissue in 2 ml of 0.01 *M* potassium phosphate buffer (pH 7.0). Two weeks after inoculation, stunted plants and plants with leaf roll or variously deformed leaves or stems were discarded. The remaining plants were reinoculated with PSbMV. Seed from the surviving plants was then planted, and the resultant plants were screened with the technique described above. Symptomless plants were indexed at bloom on *Chenopodium amaranticolor* Coste & Reyne., a PSbMV indicator local-lesion plant, that identifies possible carriers of the virus.

Inheritance of Resistance. Two resistant plant introductions, PI 368648 and PI 212610, identified in the screening test, were used as sources of resistance. 'Tekoa' and 'Precoz', two susceptible cultivars, were crossed to PI 368648. PI 368648 also was crossed to PI 212610. Each population used for the inheritance study was derived from a single selfed F_1 plant. Three F_2 populations from Tekoa × PI 368648, two F_2 populations from Precoz × PI 368648, one F_2 population from PI 368648 × PI 212610, the parental cultivars, and the individual parental PI accessions were evaluated for resistance to PSbMV. Seedlings in the four to five leaflet stage were inoculated mechanically with the virus. To reduce the possibility of escapes, inoculations were repeated 3 weeks later on all plants. After a 6-week incubation period, plants were classified as resistant or susceptible. Symptomless plants were classified as resistant (immune) and

Table 1. Chi-square analysis of F_2 populations of lentils segregating for reaction to the pea seed-borne mosaic virus.

Population no.	Cross	No. of plants observed: Susceptible	No. of plants observed: Resistant	X^2†	Probability
1	Tekoa × PI 368648	24	7	0.097	0.756
2	Tekoa × PI 368648	22	7	0.011	0.915
3	Tekoa × PI 368648	27	9	0.000	1.000
4	Precoz × PI 368648	25	8	0.010	0.916
5	Precoz × PI 368648	23	6	0.287	0.592
Total		121	37	0.211	0.646
Heterogeneity				0.195	0.660

† Expected ratio 3:1.

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Table 2. Effects of temperature and incubation time on virus symptom expression in two lentil cultivars inoculated with pea seed-borne mosaic virus.

Temperature	Days after inoculation	Plant height†		Leaf tip curling	Leaf size reduction	Rosetting	Stem bending and necrosis	Vein clearing	Tip necrosis
		Tekoa	Precoz						
18 C	7			−‡	−	−	−	−	−
	14			++	++	−	−	−	−
	21	92	89	++	++	−	−	−	−
24 C	7								
	14								
	21	76	55	++	++	−	−	−	−
				+++	+++	+	+	+	−
				+++	+++	+++	+++	++	++
30 C	7								
	14								
	21	56	44	++	++	−	−	−	−
				+++	+++	++	++	++	++
				+++	+++	+++	+++	+++	+++

† Plant height expressed as percent of uninoculated control plants. ‡ − No symptoms; + Mild symptoms; ++ Moderate symptoms; +++ Severe symptoms.

indexed on *C. amaranticolor* to verify the absence of PSbMV. Plants with questionable symptoms also were indexed on *C. amaranticolor* to aid classification into resistant or susceptible categories.

Segregation for seedcoat color in F_2 seeds was used to verify that the populations used for the inheritance study were of hybrid origin. Tekoa and Precoz have clear seedcoats whereas PI 368648 have dark striped seedcoats. The intercross of PI 212610 (red cotyledon) × PI 368648 (yellow cotyledon) was verified by segregation for cotyledon color in the F_2 seeds.

Temperature Effects. To determine the influence of temperature on disease expression, 20 virus-inoculated plants and 20 control plants of Tekao and Precoz were placed in each of three temperature control chambers set at 18, 24, and 30 C, respectively. The control plants were dusted with silicon carbide and inoculated with buffer. Each chamber had a photoperiod of 16 hours light and 8 hours darkness. Progression of PSbMV symptoms expression was noted daily. For convenience, daily observations were grouped by 7, 14, and 21 days after inoculation for the three temperatures.

RESULTS AND DISCUSSION

Lentil lines resistant to PSbMV were identified in the plant introductions. Of the lines evaluated, PI's 212610, 251786, 207745, and 368648 collected in Afghanistan, U.S.S.R., Greece, and Yugoslavia, respectively, were found to be immune to the virus. The immune response to the virus is especially valuable for breeding purposes. Immune cultivars would not be affected by virus nor be symptomless carriers capable of becoming sources of inoculum, for susceptible pea and lentil fields nearby.

A good fit to a 3:1 ratio of susceptible:resistant plants was observed in three F_2 populations from Tekoa × PI 368648 and two populations from Precoz × PI 368648 (Table 1). The observed ratios from these five populations and the total χ^2 of 0.211 with 1 df indicated that the hypothesis of a 3:1 ratio of susceptible:resistant plants should be accepted. The 3:1 F_2 ratio also indicated that resistance was recessive. The heterogeneity χ^2 showed little interaction among the five populations. Sixty F_2 plants from the intercross between the two resistant lines, PI 368648 and PI 212610, were found to be resistant to the virus, which indicated that the same recessive gene for resistance was present in the two lines. The good fit to a 3:1 ratio and the lack of segregation in the intercross (PI 368648 × PI 212610) indicated that resistance was conferred by a single recessive gene.

The identification of resistant lentil lines in the plant introductions and the indication that resistance was controlled by a single recessive gene means that resistance can be incorporated easily into local cultivars. A single recessive gene for resistance to this virus also was found in peas (2). We propose that the gene symbol *sbv* be used to denote PSbMV resistance in lentils. Thus, the genotype of the two resistant lines (PI 368648 and 212610) is *sbv sbv* and the genotype of the susceptible cultivars, Tekoa and Precoz is *SBV SBV*.

Plants infected with PSbMV were identifiable at all three temperature regimes, but symptoms of infection varied (Table 2). The first symptoms of PSbMV appeared at 30 and 24 C and included leaf tip curling and leaf size reduction. Rosetting, stem bending and necrosis, and vein clearing and tip necrosis appeared at 30 C and 24 C, but did not appear at 18 C. In the 30 C chamber, 90% of the Precoz plants and 30% of the Tekoa plants were dead at 28 days. No plants were killed at 24 and 18 C; although after 21 days at 24 C all plants exhibited the entire range of virus symptoms. Height of infected plants was reduced at all temperatures relative to that of control plants. Precoz was affected more severely than Tekoa, especially at the higher temperatures.

Knowing the effects of temperature on the expression of virus symptoms should be useful in identifying diseased plants under different temperature conditions in the field and in the greenhouse. It should also aid in classifying of resistant and susceptible plants in a breeding program aimed at developing resistant cultivars.

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