Pea Enation Mosaic Virus Resistance in Lentil (*Lens culinaris*)

H. AYDIN, Graduate Assistant, F. J. MUEHLBAUER, Research Geneticist, USDA-ARS, and W. J. KAISER, Research Plant Pathologist, USDA-ARS, Washington State University, Pullman 99164

ABSTRACT

Aydin, H., Muehlbauer, F. J., and Kaiser, W. J. 1987. Pea enation mosaic virus resistance in lentil (*Lens culinaris*). Plant Disease 71:635-638.

Twenty-nine lentil (*Lens culinaris*) plant introduction (PI) accessions or cultivars were screened for resistance to pea enation mosaic virus (PEMV). All 29 lines were susceptible and showed significant plant height and biological yield reductions when mechanically inoculated. PI 472547 and PI 472609, however, were tolerant as indicated by the lower disease scores obtained when the lines were inoculated by aphids. Also, these two lines showed significantly less reduction in plant height and biological yield attributable to virus infection than the other 27 lines. Accessions of three wild *Lens* species, *L. orientalis, L. nigricans*, and *L. ervoides*, were also susceptible when mechanically inoculated or inoculated by aphids. The tolerant lines that were identified should provide germ plasm for breeding improved cultivars.

Lentils are an important crop in the Palouse region (eastern Washington and northern Idaho) of the United States. In this area, pea enation mosaic virus (PEMV) has become a serious problem in lentil production during the last few years. Previous research in Italy (8) demonstrated that lentils are a natural host for PEMV.

PEMV is transmitted readily and efficiently by aphids (1–5,7,8), with a few isolates only transmitted mechanically (3), and is not considered seedborne. PEMV is a problem in most pea (*Pisum sativum* L.) growing areas of the United States (3). Schroeder and Barton (6) found lines of peas that were resistant to the virus but did not find immunity. They showed the resistance of G 168, a selection of peas from PI 140295, to be conferred by a single dominant gene, *En*.

Because of the serious effects of PEMV on lentil crops and because the disease appears to be a continuing problem in the Palouse region, there is a need to develop methods of control, possibly through resistant or tolerant cultivars. The objectives of this study were to identify resistance or tolerance to PEMV in available lentil germ plasm and to determine the effects of PEMV infection on plant height and biological yield of lentils.

Contribution from the ARS, USDA, in cooperation with the College of Agriculture and Home Economics, Agricultural Research Center, Washington State University, Pullman 99164. Scientific paper 7447.

Accepted for publication 24 November 1986.

MATERIALS AND METHODS

Screening with a mechanically transmissible strain of PEMV. Twenty-nine lentil lines (Table 1) that included six commercial cultivars and 23 USDA plant introduction (PI) accessions were inoculated with a mechanically transmissible strain of PEMV. The source of the virus was hairy vetch (*Vicia sativa* L.), which was infected with a mechanically transmissible strain obtained from J. R. Baggett, Oregon State University, Corvallis. The six cultivars tested were highly susceptible to PEMV under natural field infection conditions during the summer of 1983 and 1984 and were used as checks.

Seeds of the 29 lines were planted in two groups of pots during the winter of 1984–1985. Resultant plants were exposed to 24 ± 2 C and a 16-hr photoperiod in which the natural day length was extended by cool-white fluorescent lamps that provided from 180 to 230 μE m^{-2} s^{-1} at plant level. A randomized complete block design with four replicates was used. When the seedlings of each line had three sets of compound leaves, plant height was recorded and one group of plants was mechanically inoculated. The inoculum was prepared by grinding PEMV-infected faba bean (*V. faba* L.) leaf tissue in 0.01 M potassium phosphate, pH 7.0, and 0.05% Celite buffer in a mortar dusted with 600-mesh Carborundum powder. Several *Chenopodium quinoa* Willd. plants, previously held in the dark for 24 hr, were also inoculated to confirm the presence of virus in the inoculum. The second group of plants was maintained as uninoculated controls.

One week after inoculation, plant height of all plants in both groups was recorded and inoculated plants were reinoculated to minimize escapes. Plant height of all plants and disease scores of all inoculated plants were recorded at 1-wk intervals, beginning 1 wk after the second inoculation and ending with the death of inoculated plants or the maturity of either uninoculated or inoculated plants of each line. Disease symptoms were scored on a scale from 1 = no symptoms to 5 = severe infection. Two weeks after the second inoculation, each of the inoculated plants was back-indexed on *C. quinoa* to test for the presence or absence of the virus.

When the experiment was terminated, total weight of aboveground plant parts (biological yield) of inoculated and uninoculated plants was recorded.

Screening with an aphid-transmissible strain of PEMV. To screen for resistance to an aphid-transmissible strain of PEMV, the same 29 lentil lines were planted in the greenhouse in a randomized complete block design with three replicates. Three pots of each line were planted in each replicate. The greenhouse

Table 1. Lentil cultivars and plant introduction (PI) lines screened for resistance to pea enation mosaic virus

Line or identification	Origin
Brewer	United States
Laird	Canada
Tekoa	United States
Dupuy	France
Redchief	United States
Eston	Canada
PI 193549	Ethiopia
PI 212610	Afghanistan
PI 299229	Chile
PI 368648	Yugoslavia
PI 431741	Iran
PI 431742	Iran
PI 431743	Iran
PI 431744	Iran
PI 431745	Iran
PI 451763	United States
PI 471917	Canada
PI 472140	India
PI 472327	India
PI 472425	India
PI 472502	India
PI 472547	India
PI 472557	India
PI 472567	Chile
PI 472569	Egypt
PI 472583	Iran
PI 472609	Iran
PI 472636	Iran
PI 477923	*Lens nigricans* × *L. culinaris*

temperature was maintained at 21 ± 2 C and a 16-hr photoperiod in which the natural day length was extended by cool-white fluorescent lamps that provided from 180 to 230 μE m^{-2} s^{-1} at plant level.

A strain of PEMV different from that used for mechanical inoculation was used for aphid inoculations. It was obtained from field-collected faba bean plants obviously infection with PEMV, provided by J. R. Baggett, Oregon State University, Corvallis. Green pea aphids (*Acyrthosiphon pisum* (Harris)) were used as the vector, and faba beans were used to maintain both the aphids and the virus.

Four weeks after the lentil lines were planted, pea aphids were placed on infected faba bean plants and allowed to feed for 24 hr to acquire the virus. Immediately before inoculation, plant height of the lentil plants of each line was recorded and one group of plants representing all 29 lentil lines was placed in a single large cage. Aphids were removed from the faba bean plants, and 15–20 were placed on each lentil plant in the large cage. The same number of pea aphids was placed on each plant of the second group, which was then caged individually. A third group of plants was used as the uninoculated control.

After a 3-day feeding period, all plants were sprayed with diazinon to remove the aphid vectors, and the greenhouse was fogged with naled. After the insecticide applications, the plants were removed from cages and placed on greenhouse benches. One week after inoculation, plant height was recorded and the plants were scored for virus symptoms using the 1–5 scale described previously. Plant height and disease scores were recorded weekly for the inoculated and control plants. Data collection was terminated when the plants died or matured, and biological yield was determined at maturity for all plants.

Seeds of all 29 lentil lines were planted at Spillman Farm, Pullman, in the spring of 1985 to determine resistance, tolerance, or susceptibility of the lines to a natural infection of the virus. Individual plots of each line contained three 2-m-long rows spaced 30 cm apart, with the plants spaced 5 cm apart within rows. The plots were arranged in a randomized complete block design with three replicates. Seeds of the same lines were also planted at Corvallis, OR, but in single 2.5-m rows spaced 1.5 m apart and not replicated because of space limitations. Plants in the field plots were scored as a group for virus symptoms by the 1–5 scale.

Screening of wild lentil species accessions for PEMV resistance. Nine wild lentil species accessions (Table 2) representing three accessions each of *L. nigricans* (M. Bieb) Godr., *L. orientalis* (Bois Hand-Mazz.), and *L. ervoides* (Brign.) Grande were grown in the greenhouse at 21 C with a 16-hr photoperiod during the winter of 1984–1985. Treatments consisted of: 1) mechanical inoculation with the mechanically transmissible strain of PEMV; 2) inoculation with the aphid-transmissible strain of the virus, using aphids and caging all lines in a single large cage; 3) inoculation with an aphid-transmissible strain of the virus, using aphids and caging each plant individually; and 4) a set of control plants. A single plant per treatment in each of four replicates was used. Inoculation with aphids was as described previously. Two weeks after inoculation, the plants were scored for either the presence or absence of virus symptoms.

Data for all experiments were subjected to an analysis of variance, and the means were compared using the least significant difference (LSD) test.

Table 2. Wild lentil species screened for resistance to pea enation mosaic virus

Species	Collection number[a]	Origin
Lens orientalis	4	Uzbekistan, USSR
	59	Korkuteli, Turkey
	62	Gaziantep, Turkey
L. ervoides	85	Langume Forest, Turkey
	52	Sha-Hazay, Turkey
	38	Omis, Yugoslavia
L. nigricans	84	Beydap, Turkey
	25	Baska Vada, Yugoslavia
	18	El Escorial, Spain

[a]The collection number is the plant exploration number assigned when originally collected in the field, and details are on file (USDA, ARS, Pullman, WA).

RESULTS AND DISCUSSION

Screening with a mechanically transmissible strain of PEMV. All 29 lentil lines tested for reaction to PEMV were susceptible when inoculated with the mechanically transmissible strain of the virus. Typical symptoms of PEMV infection in lentils are shown in Figure 1. When the 29 lines were indexed on *C. quinoa* plants, the inoculated leaves of the indicator plants developed local lesions considered diagnostic for the presence of the virus. Plant height of infected plants was reduced compared with uninoculated control plants.

The actual reduction of plant height attributable to virus infection ranged from 31% for PI 472563 to 73% for PI 193549. Inoculated and uninoculated

Fig. 1. Pea enation mosaic virus symptoms on lentils; infected plant on left and noninfected plant on right.

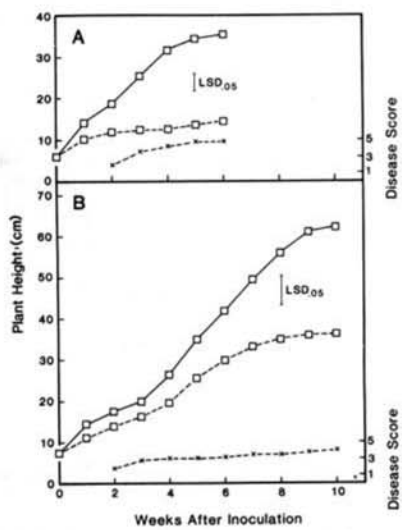

Fig. 2. Plant height of uninoculated (□—□) and mechanically inoculated (□---□) **(A)** PI 472425 and **(B)** PI 472567 and virus symptom (X---X) development. Least significant difference test ($LSD_{0.05}$) was used for comparisons within weeks.

plants did not differ in height during the first 2 wk after inoculation; however, height differences developed and increased steadily for the remaining weeks until termination of the experiment (Fig. 2). A similar pattern was observed for virus symptom development. For some of the lines, the difference in plant height for inoculated and control plants was significant 1 wk after inoculation, e.g., PI 472425 (Fig. 2A), whereas plant height differences remained small for a longer period of time for some lines, e.g., PI 472567 (Fig. 2B). There was no relation between the start of plant height reduction and final plant height reduction. Biological yield of all 29 lines was significantly reduced by the mechanically transmissible strain of PEMV and ranged from 66% for PI 472425 to 98% for Dupuy.

Screening with an aphid-transmissible strain of PEMV. Plants inoculated with aphids in individual cages developed symptoms of PEMV infection similar to those that developed in plants of the same lines that were inoculated by aphids in the single large cage. In the single large cage, the aphids had the opportunity to move to adjacent plants, whereas the aphids placed on plants in the individual cages were unable to move from one plant to another. Overall, there was no significant difference in disease scores in the two types of cages.

Plant height reduction caused by the aphid-transmitted strain of PEMV varied among lines. The percentage of plant height reduction attributable to the virus ranged from 4% for PI 472547 to 69% for PI 212610 in the large cage and from 6% for PI 472547 to 65% for PI 477923 in the individual cages. The plants that showed the least plant height reduction also had lower disease scores than other lines.

The lines that were screened showed different degrees of tolerance and susceptibility to PEMV. For example, inoculated Tekoa and Eston had disease scores close to 5 and had significant plant height reductions in response to PEMV in both types of cages (Fig. 3). PI 472547 and PI 472609 had significantly lower disease scores and only slight plant height reduction compared with other lines (Fig. 4). These two PI lines were considered tolerant to PEMV, whereas the other 27 lines were considered susceptible or very susceptible. As the experiment progressed, virus disease scores for most of the lines increased; however, disease scores for PI 472609 and PI 472547 remained constant.

The low disease scores and the lack of plant height reduction for PI 472547 and PI 472609 in response to the virus indicated that these two lines are potentially useful sources of tolerance to PEMV in lentils.

Biological yield of the individual plants of all lines was significantly reduced compared with that of uninoculated control plants (Fig. 5). Biological yield reduction in response to the virus ranged from 25% for PI 472547 to 95% for Laird in the single large cage and from 20% for PI 472547 to 96% for Laird in the individual cages. Generally, the virus caused greater percentages of biological yield reductions than of plant height reductions.

The field plot of the 29 lines at Pullman in 1985 could not be evaluated for disease scores because of minimal infection by PEMV because of a small aphid population that year. The field plot of Corvallis gave results similar to those obtained in the greenhouse. PI 472609 had the lowest disease scores, and some of the plants of that line were symptomless. PI 472547 was not as resistant as PI 472609 but appeared to be infected by an undetermined pathogen suspected of being red clover vein mosaic virus.

Both mechanically transmissible and aphid-transmissible strains of the virus caused plant height reduction and biological yield reduction in the inoculated plants; however, the mechanically transmissible strain of the virus caused greater plant height reduction than the aphid-transmissible strain. The mechanically transmissible strain used in the greenhouse experiments resulted in more severe symptoms than those we obtained in the field studies; however, the differences in symptoms may have been due to differences in virus titer.

Inoculation of the lentil accessions with the aphid-transmissible strain of the virus may be a more reliable approach than using the mechanically transmissible strain for screening for resistance or tolerance, because the symptoms produced by the former appeared similar to those observed in the field.

Two of the 29 lentil lines used in this study were considered to have a useful level of tolerance to PEMV. Those two lines, PI 472547 and PI 472609, which originate from India and Iran, respectively, are relatively shorter than the control cultivars. If these sources of tolerance were to be used in a breeding program, hybridization and selection would be required.

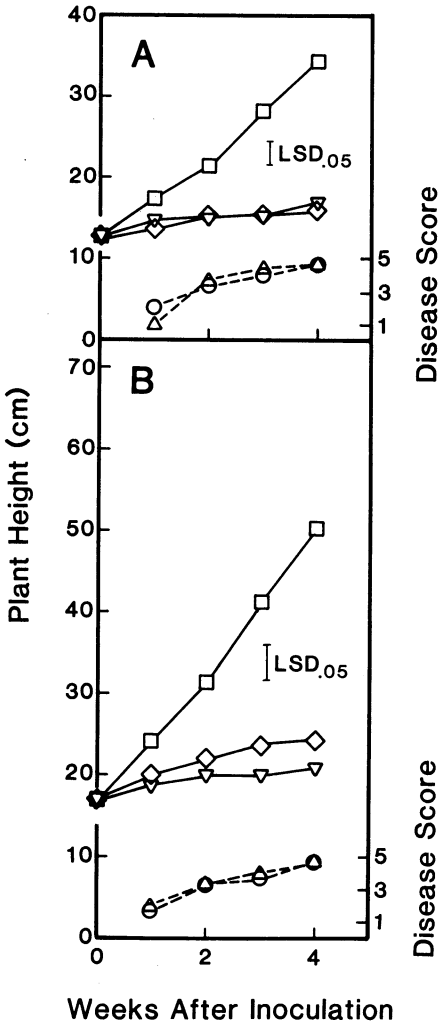

Fig. 3. Plant height of **(A)** Eston and **(B)** Tekoa inoculated with pea enation mosaic virus by aphids in a single large cage (◇—◇) and in individual cages (▽—▽) compared with uninoculated control plants (□---□). o---o = Disease scores in the single large cage and △—△ = disease scores in individual cages. Least significant difference test ($LSD_{0.05}$) was used for comparisons within weeks.

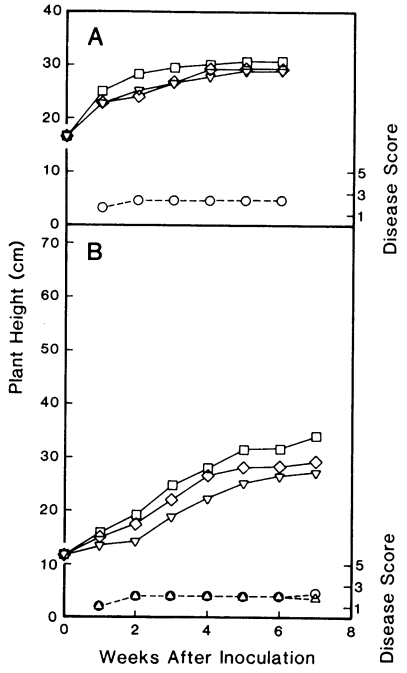

Fig. 4. Plant height of **(A)** PI 472547 and **(B)** PI 472609 inoculated with pea enation mosaic virus by aphids in a single large cage (◇—◇) and in individual cages (▽—▽) compared with uninoculated control plants (□—□). o---o = Disease scores in the single large cage and △---△ = disease scores in individual cages. Differences between control and inoculated plants were not significantly different at $P = 0.05$.

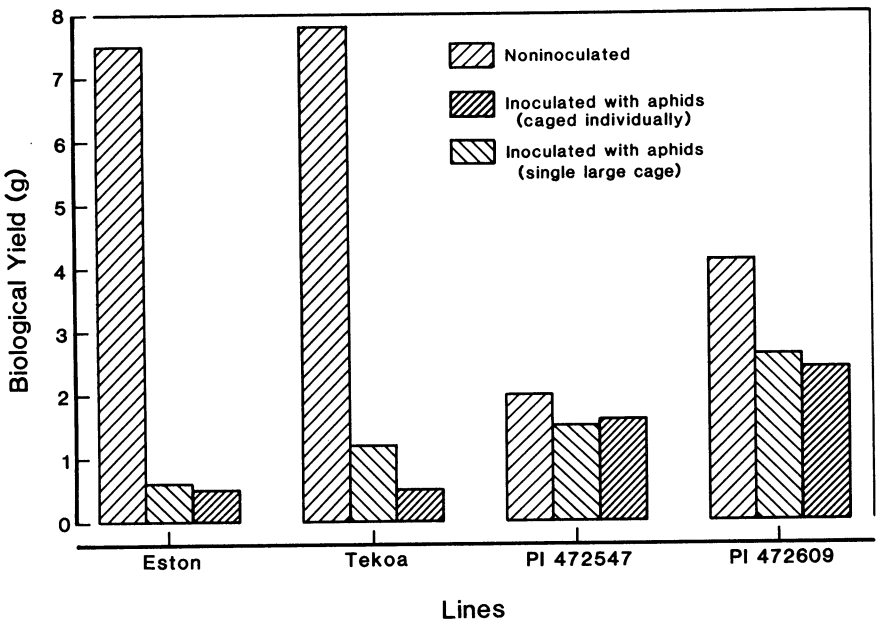

Fig. 5. Biological yield (total weight of aboveground parts) of plants inoculated with pea enation mosaic virus by aphids in a single large cage and in individual cages compared with uninoculated control plants.

It appeared that most of the other lines with some tolerance to the virus had darker green leaves, pubescent leaves and stems, were short, and had weak growth habits. PI 472609 was exceptional because it did not have pubescent leaves and had a relatively light leaf color and a vigorous growth habit. Even though it was not obvious, aphids seemingly did not prefer the pubescent lines as much as the glabrous lines.

These two tolerant lines should be used to determine the inheritance of tolerance to PEMV in lentils. The resistant pea line obtained by Schroeder and Barton (6) sometimes developed mild virus symptoms. In the case of PEMV tolerance in lentils, the plants develop mild symptoms of virus infection, making the situation similar to that in peas.

Screening wild lentil species accessions for PEMV resistance. All nine wild species accessions that were inoculated either mechanically or by aphids developed severe PEMV symptoms and, as a result, were found highly susceptible. The wild species, based on the accessions we screened, do not appear to be potential sources of PEMV resistance in lentils.

ACKNOWLEDGMENTS

We wish to thank R. M. Hannan, USDA-ARS, Pullman, WA, for providing seeds of lentil PI lines and J. R. Baggett, Oregon State University, Corvallis, for his assistance in screening the materials at Corvallis and for providing two sources of the virus.

LITERATURE CITED

1. Bath, J. E., and Chapman, R. K. 1966. Efficiency of three aphid species in the transmission of pea enation mosaic virus. J. Econ. Entomol. 59:631-634.
2. Bath, J. E., and Tsai, J. H. 1969. The use of aphids to separate two strains of pea enation mosaic virus. Phytopathology 59:1377-1380.
3. Hagedorn, D. J. 1974. Virus diseases of pea, *Pisum sativum*. Monogr. 9. Am. Phytopathol. Soc., St. Paul, MN. 47 pp.
4. Hagedorn, D. J., Layre, E. C., and Ruppel, E. G. 1964. Host range of pea enation mosaic virus and use of *Chenopodium album* as a local lesion host. Phytopathology 54:843-849.
5. Osborn, H. T. 1938. Studies of pea virus 1. Phytopathology 28:923-934.
6. Schroeder, W. T., and Barton, D. W. 1958. The nature and inheritance of resistance to the pea enation mosaic virus in garden pea, *Pisum sativum* L. Phytopathology 48:628-632.
7. Sylvester, E. S., and Richardson, J. 1966. Some effects of temperature on the transmission of pea enation mosaic virus and on the biology of the pea aphid vector. J. Econ. Entomol. 59:255-260.
8. Vovlas, C., and Rana, G. L. 1972. Le virosi delle piante ortensi in Puglia. VII. *Lens esculenta* Moench., ospite naturale del virus del mosaico con enazioni del Pisello. Phytopathol. Mediterr. 11:97-102.