PATHOLOGY AND DISTRIBUTION IN THE HOST OF PEA SEED-BORNE MOSAIC VIRUS

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Summary

Five isolates of pea seed-borne mosaic virus (PSbMV; isolates US, Q, S4, S6 and T) were compared by host range and symptomatology on 16 *Pisum sativum* cultivars and lines, 21 lines of *Lathyrus* and *Lens spp.* and several indicator species. All selections of *Pisum sativum* , except cv. Greenfeast, were susceptible to all isolates, but Greenfeast was susceptible to the isolate US . All isolates except isolate T infected the *Lathyrus* and *Lens spp.* through mechanical and aphid transmissions. *Chenopodium amaranticolor* and *Vicia faba* reacted similarly to all isolates while *Phaseolus vulgaris* cv. Hawkesbury Wonder was infected by none. The North American isolate (US) was distinguished from the Australian isolates S4, S6, Q, and T by infecting *Nicotiana clevelandii* and Greenfeast pea.

Four of the PSbMV isolates were tentatively classified using pea differentials as follows: isolates US and Q were placed in pathotype P1 and isolates S4 and S6 in pathotype P4. Using the grouping system, isolates US and Q were placed in group III and isolates S4 and S6 in group V.

The infectivity assay and double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) showed that PSbMV was present in 5 areas of South Australia, but at a low incidence (2-3%).

In all cases the highest rate of seed transmission occurred in the largest seed (83-92%) and the lowest was in the smallest seed (29-40%). Infected seed in the largest size classes was lighter in weight than the corresponding uninfected seed. Infected seed in all classes had a significantly lower germination rate than uninfected seed although the greatest reduction in germinability was in the smallest seed. In each size class uninfected seed was heavier than infected seed and germinated better.

Two-dimensional immunodiffusion tests showed that precipitin lines between all the isolates and either the US and S6 antisera were confluent with no evidence of spurs. A rapid and sensitive indirect dot-immunobinding assay (DIBA) on nitrocellulose membrane for PSbMV was developed. Non-specific binding of conjugate to the healthy antigen was partially removed by using mannose and glucose in all buffers, and completely eliminated by using either healthy plant sap, healthy seed extract or a combination of both (1:1) as the

blocking agent. The limit of detection of antigen was about 32 ng per sample. Both of the antisera detected antigen in sap extracted from peas infected with the 5 standard PSbMV isolates, as well as an additional isolate from Denmark and all isolates were detected at similar antiserum dilution endpoints.

Isolates US, Q, S4 and S6 were used in a study of the survival and partitioning of PSbMV under conditions of continuous seed transmission in the commercial pea cultivar Dundale. Under the conditions of these experiments, seed transmission was at rates exceeding 90% for all virus isolates.

Assays suitable for detecting virus in small tissue samples were used, and included DIBA with antisera to both PSbMV and cytoplasmic inclusion body (CIB) protein, and dot hybridization assay (DHA) with cDNA transcribed from virus RNA.

Virus was detectable by serology and symptoms in inoculated plants, and in all vegetative tissue of second generation (G2) plants raised from seed of the inoculated plants. However, in the third (G3), fourth (G4) and fifth (G5) sequential generations raised from seed, all plants were symptomless. Neither virus nor CIB were detectable in leaf, stem or roots by serology, but were readily detectable in some floral parts, and in immature and mature green seeds. Mature seed contained virus and CIB antigen in the testa, cotyledon and embryo. Inoculum prepared from whole seeds was infectious. The testa was shown not to be involved in transmission between generations, thus implicating the embryo alone in vertical transmission. Although virus antigen could not be detected in the emerging cotyledons of germinating seed and any true leaves by serology, the leaves contained PSbMV RNA detectable by DHA. This inability to detect PSbMV in the vegetative tissue of plants is defined as an eclipse phase.

These results show that PSbMV infection can be transferred through the vegetative phase at a subliminal level, and reaches relatively high concentrations in floral parts and seeds. Thus PSbMV may be maintained at a high level of infection in seed in the absence of any apparent symptoms in the plant, and without a requirement for horizontal transmission between plants by vectors. Such a mechanism may explain the high levels of infection commonly reported in pea breeding lines.

A study to determine whether symptomless plants exhibited cross protection showed that there was an uneven distribution of antigen after plants from G5 were challenged with the homologous isolate US. Plants in the eclipse phase in G5 thus exhibited an apparent resistance to infection by systemic movement. These results support the conclusion that peas in the eclipse phase are subliminally infected.