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Short Communication

Evaluation of USDA *Lupinus* sp. collection for seed-borne potyviruses

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Abstract

Plant viruses pose a threat to the acquisition, maintenance and distribution of lupin germplasm (genus *Lupinus*, family *Fabaceae*). The availability of sufficient quantities of healthy and virus-free seeds from maintained lupin collections is mandatory for conducting lupin research. The objective of this research was to determine which lupin species were potentially infected with potyviruses (presumably seed-borne) upon germination in the greenhouse. The procedure for screening lupin seedlings in the greenhouse for potyviruses incorporated enzyme-linked immunosorbent assay followed by elimination or segregation of infected seedlings from the population before transplantation into the field plots for regeneration and accession characterization. None of the accessions in this evaluation had been tested previously for virus. From 2002 to 2005, 15 perennial (30 accessions) and 6 annual lupin species (213 accessions) were evaluated on site at the Western Regional Plant Introduction Station in Pullman, WA, USA. While none of the greenhouse perennial seedlings tested positive for potyvirus, seedlings in three annual species (*Lupinus albus*, *Lupinus angustifolius* and *Lupinus luteus*) were infected by potyviruses, presumably by seed transmission. Future testing may focus on the annual species, thus saving limited germplasm maintenance resources.

Keywords: BYMV; ELISA; *Fabaceae*; germplasm screening; *Potyviridae*; seed-borne

Experimental

Lupin seed germination and greenhouse seedlings

Lupin seeds were scarified with a scalpel and wrapped in heavy paper treated with Captan 400-C fungicide (Bayer CropScience, Research Triangle Park, NC, USA) and then germinated for 4 d at 20°C in 100% humidity. The seedlings were planted into styrofoam flats (~7.5 cm deep) containing Sunshine® #5 soil mix (Sun Gro Horticulture Canada Ltd., Vancouver, BC, Canada). After 2–3 weeks of plant growth, all the seedlings were assayed for potyviruses by indirect enzyme-linked immunosorbent assay (ELISA) in four-plant pools. Individual plants from the tested-virus-positive pools were re-assayed. Individual infected plants were then destroyed if less than 10% of the accession population tested

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potyvirus positive. However, when greater than 10% of the plants in the accession population were infected, then the diseased plants were segregated from the healthy plants, and grown to maturity in the greenhouse. The collected seed was then denoted as 'virus-infected'. All 'virus-tested-free' seedlings were transplanted into field plots, grown to maturity, and the seeds were collected.

Enzyme-linked immunosorbent assay

Indirect ELISA assays for potyvirus group detection were conducted with reagents from Agdia Inc. (Elkhart, IN, USA) as directed with minor modifications. Briefly, 1 cm square leaf tissue from each of four seedlings and 3 ml sample extraction buffer were homogenized in a Brickman Polytron, and 100 µl of the homogenate was added to a well in a 96-well microtitre plate. After 1 h incubation, and three washes in phosphate buffered saline solution with the detergent Tween 20 (PBST buffer), 100 µl diluted (1:200) monoclonal antibody was added to each well, and held at 4°C for 20 h. The antibody was aspirated from the wells, and rinsed three times in wash buffer. Anti-mouse IgG alkaline phosphatase conjugate was diluted in IgG conjugate buffer (1:1000), and 100 µl was added to each well. The plate was incubated at room temperature for 1.5 h, and again washed three times in buffer. A 100 µl solution of *p*-nitrophenyl phosphate was added to each well, and allowed to turn colour for at least one h. The product of the enzymatic reaction was quantified by measurement at 405 in an Anthros Labtec microplate reader (Eugendorf, Austria).

Discussion

Lupins provide a variety of uses for agriculture and restoration/remediation sites. The genus *Lupinus (Fabaceae*) includes over 165 annual and perennial species which are distributed around the world from tropical to arctic climates and from sea level to alpine elevations (Gladstone *et al*., 1998).

Lupin seed acquisition, maintenance and distribution are the responsibility of the United States Department of Agriculture, Agricultural Research Service, Western Region Plant Introduction Station, at Pullman, Washington (a subset of the National Plant Germplasm System; available online only at www.ars-grin.gov/npgs). On site for distribution and maintenance are 76 lupin species of 1301 accessions. One of the most important aspects of the program is to have sufficient quantities of healthy and genetically diverse seed accessions available for research scientists (Hampton, 1983). Lupins are particularly susceptible to a number of viruses, which may adversely affect seed quality and production during seed regenerations. The first comprehensive review of destructive virus diseases of lupin included two aphid transmitted potyviruses, Bean yellow mosaic virus (BYMV) and Clover yellow vein virus (CYVV), of which BYMV is seed-borne, and not CYVV, in lupins (Jones and McLean, 1989).

Between 2002 and 2005, 15 perennial lupin species from 30 accessions did not test positive for potyviruses (Table 1(A)). The seed originated from the USA, Spain, and Mexico. In contrast, three out of six of the annual lupin species had 18 accessions with a total of 118 plants infected with potyvirus. Potyvirus-infected plants occurred in three accessions of *Lupinus albus* from Bulgaria, France and The Netherlands, and, one *Lupinus angustifolius* accession from Belarus. *Lupinus luteus* L. contained the most infected accessions, originating from the former Soviet Union, Germany, Morocco, The Netherlands, Poland, Spain and Yugoslavia (Table 1(B)). BYMV was isolated and identified from *L. luteus* tissue of potyvirus ELISA tested-positive accessions in 2005 and 2008 (Robertson, unpublished data). Historically, the first lupin species identified with BYMV was *L. luteus* in Germany (Merkel, 1929). By 1955, BYMV had been confirmed in *L. luteus*, *L. albus* and *L. angustifolius* from south eastern United States (Decker, 1950; Weimer, 1950; Corbett, 1958). Naturally infected BYMV *L. luteus* seed lots may range from 3 to 5% (Jones and McLean, 1989).

The reasons for the striking contrast between annual and perennial lupin potyvirus susceptibility are not known, but may be due to chance, genetic resistance in perennial lupin plants to potyvirus infection (undocumented), and a relatively low percentage of potyvirus seed-borne transmissions in perennial lupins.(undocumented). The majority of the lupin research involving viruses thus far has been focused on agroeconomical annual species, ignoring perennial species.

These results are the first report of the potyvirus status of *Lupinus* sp. *ex situ* germplasm. Further, the results reinforce the lupin virus prevention strategy of testing and rogueing ELISA-potyvirus-positive transplants in the greenhouse. Rogueing infected transplants will not only reduce the potential of secondary plant-to-plant spread by aphid vectors, but may also coincide with a risk of reduction in genetic diversity as found in rogueing virus-infected pea germplasm (Alconero *et al*., 1985). However, as noted by Gillaspie *et al*. (1998), seed-borne virus must be considered in the acquisition and distribution of germplasm because the virus could also cause diseases in other leguminous crops.

Table 1. Greenhouse-germinated perennial (A) and annual (B) lupin accessions assayed for potyvirus by enzyme-linked immunosorbent assay using a monoclonal group-specific antibody

Taxon	Number of accessions infected[a]	Number plants assayed
(A) Perennial		
Lupinus polyphyllus Lindl.	0/3	232
Lupinus rivularis Dougl. ex Lindl.	0/2	271
Lupinus sericeus Pursh	0/2	252
Lupinus sulphureus Douglas ex Hook.	0/2	166
Lupinus albicaulis Douglas	0/2	180
Lupinus albifrons Benth.	0/2	113
Lupinus arbustus Douglas ex Lindl.	0/3	144
Lupinus argenteus Pursh	0/4	455
Lupinus bicolour Lindl.	0/1	67
Lupinus elegans Kunth	0/1	132
Lupinus formosus Greene	0/1	130
Lupinus latifolius Lindl. ex J.G. Agardh	0/1	83
Lupinus lepidus Douglas ex Lindl. var. *aridus* (Douglas) Jeps.	0/2	149
Lupinus leucophyllus Dougl. ex Lindl.	0/3	250
Lupinus littoralis Douglas	0/1	77
Total	0/30	2701
Total perennial species tested: 15		
Average plant number tested per accession: 90		

	Number of infected accessions[a]	Percent of infected accessions	Number of plants infected[b]	Percent of infected plants	Average percent of infected plants
(B) Annual					
Lupinus albus L.	3/53	5.66	20/6021	0.33	4.89
Lupinus angustifolius L.	1/61	1.64	4/6409	0.06	2.77
Lupinus luteus L.	14/38	36.84	164/4247	3.86	7.77
Lupinus mutabilis Sweet	0/58	0	0/4488	0	0
Lupinus hispanicus Boiss. & Reut	0/1	0	0/119	0	0
Lupinus mexicanus Cerv. ex Lag.	0/2	0	0/196	0	0
Totals	18/213	8.45	188/21,480	0.88	
Total number of annual species: 6					
Average plant number tested per accession: 100					

[a] Numerator = number of potyvirus-infected accessions, denominator = total number of accessions.
[b] Numerator = number of potyvirus-infected plants, denominator = total number of plants.

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