Tropaeolum Mosaic Potyvirus (TropMV) Reduces Yield of Andean Mashua (*Tropaeolum tuberosum*) Accessions

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Abstract. Quechua farmers have cultivated mashua (*Tropaeolum tuberosum* Ruiz & Pavon) and other tuber crops for thousands of years. The practice of trading seed tubers may have contributed to dispersal of viral diseases, such as the tropaeolum mosaic virus (TropMV). We surveyed 17 accessions of mashua collected from Quechua farmers in the provinces of Cuzco and Ayacucho, Peru. Most cross-reacted with the TropMV antibody and showed viral disease symptoms. Significant differences were observed between accessions from Cuzco and Ayacucho, with respect to virus infection and tuber yield under greenhouse conditions. Of the accessions from Cuzco, 87% displayed viral symptoms, while only 22% from Ayacucho showed symptoms. Fewer tubers from Cuzco generated mature plants. In turn, those mature plants produced lower tuber yields. The practice of trading seed tubers may be advantageous for promoting crop diversity but can be harmful when diseased seed tubers are being traded. A program to generate and distribute virus-free seed tubers among Andean farmers would contribute to higher crop yields while preserving local customs and crop diversity.

Mashua (*Tropaeolum tuberosum* Ruiz & Pavon), a member of the Tropaeolum (Nasturtium) family, is one of the most intensively cultivated root and tuber crops in the Andean region. Mashua tubers are prized for their mustard-like flavor. The tubers are very nutritious, with high levels of ascorbic acid, proteins and starch (Flores et al., 2003). Andean farmers cultivate a great diversity of mashua cultivars at elevations of 2,000 to 4,000 m above sea level. Along with potato, mashua is one of highest yielding Andean root and tuber crops. It is also appreciated for its medicinal, nematicidal and insecticidal properties (Arbizu and Tapia, 1994; National Research Council, 1989). Reduced interest in growing mashua in recent years has been ascribed to great losses in productivity, which are attributed to viral diseases (CIP, 1994; National Research Council, 1989).

The Tropaeolum mosaic potyvirus (TropMV) appears to be a major disease of mashua. Delhey and Monasterios (1977) were the first to report a mosaic disease of mashua in Bolivia, but no identification was made. More recently, Soria et al. (1998) assigned the name tropaeolum mosaic virus to the causal agent of a mosaic disease of mashua, and serological features indicated that the virus may belong to the potyviridae family.

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These authors reported the disease symptoms as vein clearing, leaf distortion and necrosis. TropMV was isolated from mashua, but other plant species such as *Nicotiana benthamiana*, *N. debneyi*, *N. rustica*, *N. occidentalis*, *Gomphrena globosa* and *Chenopodium quinoa* may be mechanically infected by the virus (Soria et al., 1998). Tropaeolum mosaic virus, sometimes called nasturtium mosaic virus, also infects *Tropaeolum majus* and *Zinnia elegans* and has been detected in the United Kingdom (Smith, 1950), U.S. (Jensen, 1950), South Africa (Da Graça and Martin, 1977), and different regions of South America (Silberschmidt, 1953; Soria et al., 1998). Transmission is thought to occur via aphids (Jensen, 1950).

Most mashua accessions studied at the Santa Catalina Experimental Station in Quito, Ecuador, were infected with TropMV (Soria et al. 1998). It is possible that many of the

cultivars used by the Andean farmers are virus-infected and that infected seed tubers might have been propagated for centuries, leading to lower crop production in the high Andes. Indian groups, such as the Quechuas, have long traded mashua tubers and other crops in local markets. This custom may have contributed to the dissemination and perpetuation of viral diseases. We hypothesized that mashua seed tubers collected from Quechua farmers may be contaminated with TropMV virus, which in turn results in lower productivity. To test our hypothesis, we surveyed 17 mashua accessions from local Quechua markets in Peru for TropMV and evaluated their tuber yield under greenhouse conditions.

Materials and Methods

Plant material. Tubers were collected between 1997 and 1998 in Cuzco and Ayacucho, Peru, from grower markets in the communities of Ccorao, Chawaitiri and Paruro (Cuzco) and from San Jose de Arizona (Ayacucho) (Table 1). Cuzco city is at an elevation of 3400 m and Ayacucho city is at 2800 m. The tubers were brought to Pennsylvania State University and accession letters were assigned to each morphotype based on origin and Quechua farmers' description (Table 1). Tubers were either stored at 4 °C until they began sprouting, or frozen for subsequent ELISA tests and protein analysis.

Tuber yield assessment. After incubation at 4 °C for about 8 d, the sprouting tubers were planted in 10-L pots, one tuber per pot, containing an all purpose commercial potting soil mixture (Schultz Co., St. Louis, Mo.). Plants were grown in greenhouse benches under natural light levels at temperatures ranging from 16 to 28 °C. Plants were manually watered two times a week with no addition of fertilizer to the pots. Because the plants were very susceptible to white flies, thrips and aphids, they were sprayed with a dilute solution of orthene, 3.8L/9.3 m², every 2 weeks to control insect pests.

Experiments were designed as two randomized blocks with five pots of each accession per block. Plants were planted in July of 1998 and tuberization occurred between November and February. Tubers were collected in February and nondestructively analyzed for tuber yields.

Table 1. Mashua accessions from several regions of Peru and described by local common names.

Mashua accession	Quechua name	Origin (Community)
AA	Yana ñahui	San Jose de Arizona, Ayacucho
BDE	Yana ñahui	San Jose de Arizona, Ayacucho
CC	Yana aña	San Jose de Arizona, Ayacucho
GG	---	San Jose de Arizona, Ayacucho
HH	Cheeje che aña	San Jose de Arizona, Ayacucho
II	Huashkar aña	San Jose de Arizona, Ayacucho
MN	Huacastia aña	San Jose de Arizona, Ayacucho
NN	---	San Jose de Arizona, Ayacucho
S/C	Q'ellu	San Jose de Arizona, Ayacucho
Acu	Yana ñahui	Ccorao, Cuzco
Bcu	Puca aña	Ccorao, Cuzco
Dcu	Zapallu aña	Ccorao, Cuzco
Fcu	Wakak	Chawaitiri, Cuzco
Jcu	Papa aña	Ccorao, Cuzco
Kcu	Yana	Ccorao, Cuzco
Ocu	Puca ñahui	Paruro, Cuzco
Qcu	Yacuar huacac	Ccorao, Cuzco

²No name recorded.

The number and fresh weight of tubers were determined for each accession. Statistical tests included analysis of variance (ANOVA) using the general linear model procedure, and regression analysis using the regression procedure of SAS statistics software (SAS Inst., Cary, N.C.).

Virus inoculations and plant analysis. Frozen TropMV infected tobacco leaves were kindly donated by the American Type Culture Collection (Rockville, Md.). For inoculations we thawed TropMV infected tobacco leaves and homogenated in 0.1 M sodium citrate buffer, pH 7.4; at a ratio of one gram of tissue per 50 mL of buffer. This solution was applied on leaves previously dusted with 600-mesh carborundum, as described by Soria et al. (1998). Viral solutions were kept on ice until inoculation. TropMV was inoculated on tobacco (*Nicotiana benthamiana*), which was used as a positive control for the ELISA tests, and mashua accessions AA, MN, GG, Acu, and S/C, which were used for visual comparison against un-inoculated plants.

Protein extraction and ELISA. Thirteen accessions of mashua brought from Peru were used for ELISA tests. Each sample used for ELISA tests contained the homogenate of at least 3 different tubers of the same accession. Soluble proteins from tubers were extracted according to the method described by Savary and Flores (1994). After precipitation with cold acetone overnight, proteins were centrifuged for 20 min at 10,000 g_n and resuspended in Tris buffer (50 mM Trizma, pH 8). Protein concentration was determined by the Bradford assay (Bradford, 1976).

A monoclonal antibody raised against the TropMV was kindly donated by T.A. Evans, Department of Plant and Soil Sciences, University of Delaware. ELISA tests were done as described by Guimarães et al. (2001). Detection was performed using a chicken anti-IgG coupled to alkaline phosphatase, and the p-nitrophenylphosphate as a chromogenic substrate (Sigma, Saint Louis, Mo.). Readings were done with a SpectraMax Plus (Molecular Devices, Sunnyvale, Calif.) microplate reader at 405 nm. ELISA tests were replicated twice.

Results

Tubers of mashua from four Quechua communities were evaluated for the presence of TropMV. Disease symptoms (Fig. 1) include vein clearing, leaf and stem distortion followed by necrosis and reduced growth after a 4-week period (Soria et al., 1998). Various levels of infection were detected by ELISA tests of tubers of 13 accessions. (Fig. 2). All accessions reacted with the TropMV monoclonal antibody, suggesting that most mashua accessions from Quechua farmers have some viral contamination. Tubers were induced to sprout and plants were non-destructively evaluated for the presence of the virus. Table 2 shows the survival rate of plants and the incidence of virus. Accessions from Cuzco had a higher incidence of disease symptoms and lower survival rates than those from Ayacucho. There was significant relationship when tuber-seed survival was regressed on disease symptom ($r^2 = 0.45^{***}$, $y = 1.02 - 0.298x$). Only 52% of accessions from Cuzco survived to maturity, of which 87% displayed visual viral symptoms.

In contrast, 95% of the tubers from Ayacucho produced mature plants and only 22% of those had observable disease symptoms.

Those plants that reached maturity were evaluated for tuber yield. Average tuber weight, number of tubers, and total tuber weight produced per plant varied greatly among the different accessions (Table 3). Accessions were found to differ significantly in total tuber weight ($P < 0.0001$) and tuber number ($P < 0.0008$). Accessions Ocu and BDE produced more tubers per plant but these tubers were small 2.8 g/tuber and 3 g/tuber, respectively. In contrast, accession MN produced the largest and heaviest tubers (9.7 g/tuber). Accessions HH and Kcu failed to produce any tubers. Most of the accessions produced abundant foliage in the greenhouse with the exception of BDE, Fcu, Dcu, and Kcu,

which showed viral disease symptoms. Viral contamination may explain the lower number of tubers and lower tuber weight found in these accessions. Tuber weight regressed on disease symptom severity was highly significant ($r^2 = 0.25^{***}$, $y = 11.07 - 4.114x$). The average tuber weight of accessions from Ayacucho was 11 g while accessions from Cuzco averaged 7 g, of those plants that produced tubers. There was no significant relationship between tuber number and disease severity.

Discussion

The full spectrum of viral diseases of mashua is not known. Potyvirus is the only class of viruses that has been reported from mashua isolates. Recent attempts to identify viral dis-

Fig. 1. *Tropaeolum mosaic potyvirus* (TropMV) symptoms in mashua. (A) Mashua infected with TropMV, arrow points to diseased upper leaves. Stunted stems, vein clearing and downward curling of leaves are symptoms of TropMV disease. (B) Uninfected mashua, arrow points to healthy upper leaves.

Fig. 2. *Tropaeolum mosaic potyvirus* (TropMV) titer of 13 mashua accessions as revealed by ELISA. The TropMV antibody was used at a titer of 1:1000. *Nicotiana benthamiana* infected with TropMV was used as a positive control [Nicotiana (+)]; and uninfected *N. benthamiana* as a negative control [Nicotiana (-)]. The negative control was used as a baseline and all absorbances were subtracted from that. Values are means of three tubers $\pm$ SE.

eases of mashua pointed to two potyviruses, *tropaeolum mosaic potyvirus* (TropMV) (Soria et al, 1998) and *potato virus T* (PVT) (Lizárraga et al., 2000). About 74% of the mashua accessions studied by Soria et al. (1998) from the Santa Catalina Research Station in Quito, Ecuador, were infected by TropMV. It is possible that TropMV has been widely spread throughout the Andes via infected seed tuber. In this paper we demonstrated that the majority of mashua accessions from four Quechua communities showed viral disease symptoms and 13 accessions reacted with the TropMV antibody.

Variation was observed between the accessions from Ayacucho and Cuzco. Fewer tubers from Cuzco produced mature plants than those from Ayacucho. Of the mature plants, more from Cuzco showed viral symptoms than from Ayacucho. Tuber yields were lower for accessions from Cuzco than those from Ayacucho, averaging 7 vs. 11 g per plant, respectively. Causes for such significant variation between mashua accessions from Cuzco and Ayacucho are not entirely understood. It was difficult to

exactly simulate day length in a Pennsylvania greenhouse to Andean conditions.

Availability and quality of seed tubers vary by season and year due to environmental changes. The socioeconomic status of communities also has a noteworthy impact on the diversity of tuber crops. Those communities made up of less affluent families are likely to have limited and lower quality accessions of mashua, or other root and tuber crops. After studying the social and ecological settings that determine diversity of native crops in the region of Paucartambo, Peru, Zimmerer (1996) discovered that the wealthier farmers maintain greater crop diversity than poorer farmers. Another study performed in the region of Picot, Peru, showed that farmers' approaches to obtaining new seed tubers vary according to socioeconomic status (Bianco and Sachs, 1998). Wealthier families purchase seed tubers at the markets in Cuzco city or in the smaller towns of Pisac and Chinchero. In contrast, poorer families generally exchange seeds for their labor. Acquiring better quality seed tubers is a major concern for Andean farmers. Although families save their seed tubers from year to year, they need to replenish their genetic stocks from other farmers or purchase them at local markets and annual seed fairs every 4 or 5 years (Bianco and Sachs, 1998).

Viral diseases are known to cause great yield losses in potatoes and other root and tuber crops (Ngeve and Bouwkamp, 1991; Lot et al., 1998; Hamm and Hane, 1999). A comparative study on 'Russet Norkotah' potato using PLRV diseased seed tubers versus virus-free seed tubers showed 60% yield reduction and 88% decrease in marketable yields in the diseased plants (Hamm and Hane, 1999). In this paper we showed a significant relationship between tuber weight and disease symptoms in greenhouse conditions. We found significant differences in tuber weight and tuber quantity among accessions, suggesting that resistance to viral diseases may exist. Such diversity represents valuable resources for breeding purposes. A field trial using disease-free seed tubers is necessary to show the extent of viral disease damages on mashua yields in situ, and to account for morphological variations among accessions.

Viral diseases in seed tubers are not necessarily noticeable, unless biochemical tests are

performed. We did not observe any detectable changes in tuber morphology in greenhouse conditions. Viral symptoms were only noticeable after sprouting. The use of virus-free seed tubers could be of major importance to Andean farmers. Institutions such as CIP (International Potato Center) and CIAT (International Center for Tropical Agriculture) have recommended the use of virus-free seed tubers and the distribution of decontaminated germplasm accessions (CIP Annual Report, 1994). However, technology for producing virus-free mashua seed tubers has not yet been readily accessible to Andean farmers.

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Table 2. Mashua tuber viability and subsequent *tropaeolum mosaic potyvirus* (TropMV) viral symptoms in foliage.

Mashua accession	Tubers that produced plants (no.)	Visual virus symptoms ^z
AA	100	—
BDE	100	+
CC	66	+
GG	100	—
HH	100	—
II	80	—
MN	100	—
NN	100	—
S/C	100	—
Acu	100	—
Bcu	20	+++
Dcu	60	+
Fcu	60	++
Kcu	33	++
Jcu	20	+++
Ocu	100	+
Qcu	20	+++

^z(—) No symptoms; (+) one or two stunted stems, clear veins and curly leaves per plant; (++) less than 50% stunted stems, clear veins and curly leaves per plant; (+++) necrotic leaves, more than 50% stunted stems, clear veins and curly leaves per plant.

Table 3. Tuber yield for different accessions of mashua grown in pots in the greenhouse.

Mashua accession (SE)	Avg tuber wt (g)/plant (SE) ^z	Avg no. of tubers/plant (SE)	Total tuber wt (g)/plant
AA	3.7 (±0.2)	3.0 (±1.1)	11.2 (±0.6)
BDE	3.0 (±1.0)	6.0 (±2.1)	15.0 (±0.6)
CC	3.4 (±0.0)	1.0 (±0.0)	3.4 (±0.0)
GG	5.5 (±0.3)	1.8 (±0.3)	9.9 (±0.7)
HH	---	---	---
II	2.0 (±0.0)	0.3 (±0.3)	0.6 (±0.1)
MN	9.7 (±0.2)	3.0 (±0.0)	29.0 (±0.7)
NN	6.3 (±0.0)	0.5 (±0.5)	3.1 (±0.4)
S/C	6.1 (±0.1)	2.3 (±0.3)	14.0 (±0.5)
Acu	3.9 (±0.4)	3.0 (±0.7)	11.7 (±0.1)
Dcu	2.1 (±0.1)	2.0 (±0.6)	4.1 (±0.2)
Fcu	1.3 (±0.0)	1.0 (±0.0)	1.3 (±0.0)
Kcu	---	---	---
Ocu	2.8 (±0.1)	4.0 (±1.0)	11.1 (±0.9)

^z±SE, n = 4.

^yNo tuber formation.