

THE STATUS OF *POTATO LEAFROLL VIRUS* IN KENYA

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SUMMARY

A survey of potato leaf roll disease conducted in major potato growing regions of Kenya (Meru, Kiambu, Nyamira and Kisii districts) revealed the highest incidence in Kisima location, Timau division of Meru district and an absence of PLRV in leaf-rolled samples from Githongo location of the same district. The lowest incidence was recorded in Kisii district. The virus was detected in the phloem tissues of potato sprouts and leaves by tissue blot immunoassay (TBIA). The method was found to be simple, rapid and sensitive in detecting PLRV in infected potato plants. In *Myzus persicae* Sulz., the Acquisition Access Period (AAP), Inoculation Access Period (IAP) and the Latent Period of PLRV, isolated from potato variety 'Pimpernel' collected from Tigoni location, Kiambu district, were typical of PLRV. It is suggested that virus control in potato in Kenya can be made more effective by planting virus-free seed-tubers and by early treatment with insecticides. Additionally, volunteer plants and alternative hosts should be rogued and destroyed because they serve as sources of virus for subsequent infections.

Key words: potato, PLRV, tissue blot immunoassay, aphid transmission, control.

INTRODUCTION

Potato leafroll virus (PLRV) is a very important pathogen of potato due to the large yield losses it induces wherever potatoes are grown (Peters and Jones, 1981). According to farmers and potato researchers, the number of potato varieties grown in Kenya has declined because of the selection of varieties that exhibit superior qualities and the elimination of others that have degenerated. Furthermore, it has been recognised that potato degeneration in areas afflicted with the disease can be avoided by using seed tubers obtained from upland areas or from a seed certification unit, a method used elsewhere for many years (Robert *et al.*, 2000). But potatoes

so obtained become curled (infected) after two to three seasons due to a rapid spread of PLRV by aphid transmission. Aphids transmit the virus in a persistent manner and the green peach aphid (*M. persicae*) is the most efficient vector (Harrison, 1984).

PLRV is the only potato virus that has been successfully controlled by the use of insecticides because of its persistent (long term) transmission. Measures to control field spread of PLRV take advantage of the latent period by utilising insecticides to kill the aphid apterae before they inoculate the virus to healthy plants. However if alate aphids arrive already carrying the virus from other infection sources such as ware potatoes, then insecticides cannot be relied upon to prevent spread.

Surveys of PLRV have been done extensively in North America and Europe (Ellis and Stace-Smith, 1993), but a number of tropical countries (Kenya included) have not been surveyed. Here we report the results of surveys for the disease and its distribution in major potato growing areas of Kenya.

MATERIALS AND METHODS

Surveys for potato leafroll disease were conducted in fields, selected from areas with diffuse and intense potato production (Fig. 1 and Table 1). The districts of Meru and Kiambu in Eastern and Central provinces, respectively, were chosen to represent areas with intense potato production while those of Kisii and Nyamira in Nyanza province, areas with diffuse production. Surveys were conducted during the potato-growing seasons of 1996 and 1998 by walking through potato fields and examining potato plants for the presence of leafroll symptoms (visual inspection). Disease incidence was assessed as the number of infected plants in each plot expressed as a percentage of total number of plants observed. Five sites per field were randomly selected to give a representative figure. At every site an area of 5 m by 30 m was evaluated. Where rows were less than 30 m long, the length of the rows was used. Leaf samples of diseased plants were put in paper bags and shipped to the laboratory for further virus analysis by TAS-ELISA, performed essentially as described by Muniyappa *et al.* (1991). Quantitative measurements of generated *p*-nitrophenol were made by determining absorbance at 405 nm (A_{405}) in a Titertek® MCC/370 model spectropho-

Table 1. Results of a survey for PLRV infection in major potato growing locations of Kenya: estimated incidence by visual and ELISA analyses.

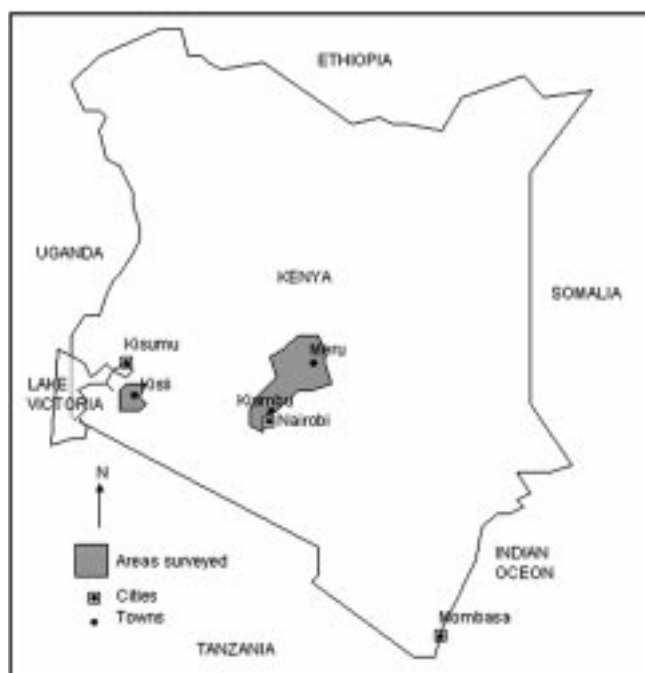
Location	No. of surveyed farms	No. of ha covered	Mean disease incidence	
			Visual	Elisa
Ngecha	3	5	16.1	15.2
Tigoni	5	5	3.1	2.3
Muguga	3	5	14.0	14.0
Uplands	6	5	10.3	9.7
Ndenderu	3	5	14.5	13.0
Kibirichia	4	7	6.5	4.3
Ntugi	4	5	1.8	1.1
Githongo	2	3	0.3	0.0
Katheri	4	3	5.3	5.2
Kisima	20	9	28.9	25.3
Kirimara	7	8	25.8	20.0
Nyaribari Masaba	11	11	3.2	2.8
Nyaribari Central	6	6	3.1	3.1
Kitutu Chache	3	4	1.5	1.0
Nyaribari Chache	5	4	1.6	1.1
Kitutu East	5	6	6.4	5.6

tometer (Labsystem Co., Finland). Twice the healthy control absorbance was used as the threshold for determining PLRV infections.

For the detection of PLRV in potato plant tissues, a modified method of the tissue blot assay (TBIA) developed by Lin *et al.* (1990) was used. All incubations were done at room temperature with agitation. Nitrocellulose (NC) membranes (0.45 µm pore size; Schleicher and Schuell, Dassel, Germany) were marked with a water-proof pen into square grids equivalent to the size of plant material to be blotted. Just before blotting, the membranes were wetted briefly in a 2.5% Na₂SO₃. Fresh plant tissue was cut with a sharp blade, which was washed with 70% alcohol after every cut and the newly cut surfaces immediately pressed firmly but gently onto the membrane. The unoccupied protein-binding sites on the membranes were blocked by immersing the membranes in phosphate-buffered saline (PBS) (per liter: 8.0 g NaCl, 0.2 g NaN₃, 0.2 g KCl, 1.44 g Na₂HPO₄·2H₂O, 0.2 g KH₂PO₄, pH 7.4) containing 1 µg ml⁻¹ polyvinylalcohol (PVA) 72000 (Merck, 821038) for 1 min. The membranes were then washed for a few seconds in PBS-Tween (per liter: PBS, 0.05% Tween 20) and the detecting monoclonal antibody (Mab) specific to the virus diluted 1:200 in conjugate buffer [per liter: PBST, 20 g PVP 10,000 (Mr), 0.2% egg albumin) was added. After incubation for 1 h, the membranes were washed twice (each, 10 min) in PBST before alkaline phosphatase-labelled rabbit-anti-mouse diluted 1:1000 in conjugate buffer was added, and the membranes were incubated for 40 min. After a further two washes in PBST as above, the membranes were pre-equilibrated for 5 min in Tris-buffered saline (TBS) buffer (0.2 mM Tris-HCl, pH 8.0, 2.0 mM MgCl₂) and then developed for 10 min in freshly prepared substrate mixture (1:1) of solution A [Naphtol-AS-MX phosphate sodium salt (Sigma, St. Louis, USA) in distilled water 6

mg/15 ml] and solution B [Fast Red TR-salt (Sigma, St. Louis, USA) in TBS, pH 8.6, 90 mg/15 ml]. To stop the reaction, the membranes were rinsed briefly in water and dried between sheets of Whatman 3MM paper. For the interpretation of the results the membranes were observed under a stereo-microscope (Stemi 2000-C, Zeiss, Germany).

Plant tissues infected with the virus were clearly identified using a combination of this method and a dissecting microscope. PLRV was detected by the pinkish/reddish colour stain in vascular tissues. The virus was mostly detected at the bases of the plant tissues analysed. Its amount in different parts of the plant was determined by

**Fig. 1.** Map of Kenya showing areas where potato samples with PLRV symptoms were collected.

assessing the colour intensity in vascular tissues. No PLRV was detected in tissues other than the phloem.

RESULTS AND DISCUSSION

A total of 321 samples from 91 fields were collected and analysed in the laboratory by ELISA and TBIA. Of these, 287 were found to be PLRV-positive. Virus was present in all the four districts surveyed. Disease incidence was very high in Timau division of Meru district (Table 1), with Kisima location showing the highest incidence followed by Kirimara location. The fields with the highest leafroll incidence generally grew varieties like Kerr's pink, Ngure (Pimpernel) and Annett. PLRV was not detected in leaf-rolled plants from Githongo location of Abuthoguchi West division (where potatoes are grown under sprinkler irrigation). In Kiambu district, Ngecha location of Limuru division had the highest incidence of potato leafroll disease; the disease was present on every farm that was visited in this district. In Nyamira district, the highest leafroll incidence was in Kitutu East location of Rigoma division. Kisii district had the lowest leafroll incidence (uniformly distributed in all potato growing locations). Although leaf-rolled plants were found on all farms visited, the virus was detected in few samples.

In all districts, disease incidence was dependent on potato variety grown and source of seed tubers. In Kisii, farmers weed the crop only once and a variety of weeds were present as compared to Meru and Kiambu.

The AAP, IAP and Latent periods of PLRV (from cv Pimpernel from Tigoni) were assessed essentially as described by Bakker (1974) and Gildow (1982) and found to be similar to those obtained by earlier workers (Sugawara and Kojima, 1973; Syller, 1994; Singh *et al.*, 1995).

It was evident from survey results that disease incidence was high in areas with low rainfall and high temperatures. Thus, our findings agree with those of Raman (1980) who observed that low rainfall and high temperatures are ideal for high aphid multiplication and movement. The failure to detect PLRV in leaf-rolled samples from Githongo location of Meru district suggests that the symptoms can be attributed to causes other than PLRV infection. For example Gibson (1974) observed that feeding by non-viruliferous *Macrosiphum euphorbiae* induced rolling of upper leaves (top-roll) in potato plants. Since *M. euphorbiae* is the most abundant aphid in Kenya (Eastop, 1953; Were *et al.*, 1996), it is highly likely that the leaf-rolled samples that were PLRV-negative were collected from plants that had been heavily infested with the aphid.

The high incidence of PLRV infection in districts of Meru and Kiambu can be attributed to the fact that most farmers intercropped potatoes with such crops as maize, beans, or collards, which can act as alternative hosts for the aphids. The intercrops also facilitate movement of aphids from plant to plant thus helping to

spread the aphids as well as the virus (Eastop, 1953). Moreover, farmers in the survey area do not uproot volunteer crops of potato and these can serve as virus reservoirs. Areas with low PLRV incidences either had high rainfall or potato was grown under sprinkler irrigation and in pure stands, which reduced the movement of aphids. Virus spread in these areas can be attributed to the planting of infected seed tubers. It was observed that farmers grow their own seed because seed production programs have excluded popular varieties preferred by farmers. In farms using certified seeds, disease incidence was low. In areas where weeds such as *Datura stramonium*, *Physalis floridana*, *Amaranthus* sp. and *Solanum nigrum* were very common, the incidence was high probably because these weeds can be hosts for both the aphids and the virus (Tamada *et al.* 1984).

TBIA results show that the method can easily be used to locate PLRV in plant tissues. PLRV was detected only in phloem tissues of potato sprouts and leaves. These findings agree with those of Richard *et al.* (1993), which confirmed earlier work that detected virus particles in the phloem tissues by electron microscopy of thin sections (Kojima *et al.*, 1969) and by fluorescent light microscopy of sections stained with fluorescent-conjugated antibody to PLRV (Barker and Harrison, 1986). Since it is the easiest way to locate viruses in plant parts and tissues in Kenya, TBIA can be very useful during surveys. Samples can be blotted on paper in the field and shipped to the laboratory in envelopes for analysis thus cutting on transport costs of bulk material. The method was found to be sensitive, rapid, cheap and more convenient than ELISA.

In conclusion, *Potato leafroll virus* continues to be a major source of yield reduction in Kenya and because most farmers cannot afford pesticides to control aphids, use of virus-free seed remains the most important control measure. For this reason, suitable and efficient seed production programmes should be devised for all potato-growing regions. Additionally, a cheap and effective system of insect monitoring should be developed especially in developing countries to give warnings of insect outbreak to farmers. Basky (2002) found that PVY and PLRV infections remained below 5.2% and 1.7%, respectively, if the cumulative vector intensity did not reach a value of 10. This means that for effective control of PLRV, potato fields should be sprayed with insecticides at the first warning of the build-up of vector aphid populations. However, to buy insecticides involves spending money, which most potato farmers in Kenya do not have. We therefore recommend the roguing and destruction of volunteer plants and alternative virus hosts because they serve as sources of virus for subsequent infections.

ACKNOWLEDGEMENTS

We thank Dr. Stephan Winter for providing PLRV monoclonal antibodies, and Dr. Hut for his assistance in

the interpretation of TBIA results.

This work was funded by the German Academic Exchange Service (DAAD) and the Dean's Committee Grant of the University of Nairobi, Kenya.

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Received 11 April 2003

Accepted 17 July 2003