Use of Thermotherapy to Free Potato Tubers of Alfalfa Mosaic, Potato Leaf Roll, and Tomato Black Ring Viruses

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ABSTRACT

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Alfalfa mosaic virus (AMV), potato leaf roll virus (PLRV), and tomato black ring virus (TBRV) were eliminated from diseased tubers of several potato (*Solanum tuberosum*) cultivars by hot-air treatment at 37 C for 3–6 wk. This treatment also eradicated viruses in tubers dually infected with PLRV and TBRV. Virus could not be detected in plants grown from these tubers following repeated indexing by mechanical means and by serology (AMV and TBRV) or by aphid-transmission tests (PLRV); and tubers harvested from these plants produced normal plants that also indexed negative for virus infection. Similar hot-air treatments for up to 10 wk did not eradicate two strains of potato virus Y. Hot-water treatment at 50 C for 25–180 min or 52.5 C for 15–90 min did not free tubers of AMV, PLRV, and TBRV. Survival of tubers of eight potato cultivars in various hot-air treatment tests after 6 wk ranged from 44 to 87%. Hot-air treatment eliminated PLRV from tubers of over 50 imported and Kenyan potato lines. Potato cultivars freed of virus infection by thermotherapy have been distributed to various potato improvement programs in East Africa.

Additional key words: East Africa, potato viruses.

The potato (*Solanum tuberosum* L.) is an important crop in the highlands of East Africa (Kenya, Tanzania, and Uganda) (1). In Kenya, the government is striving to increase potato production by introducing higher-yielding cultivars that also have resistance to important pests and diseases. Potato germplasm, mainly in the form of tubers, which are notorious as a reservoir for numerous diseases that affect the crop (3,12), was imported from various countries through the Plant Quarantine Station (PQS) at Muguga. There the tubers were sprouted and indexed for different pathogens, particularly viruses. Only tubers or rooted cuttings from healthy plants (not the original mother plants) were released to the Kenya Potato Research Station at Tigoni for use in its research and production programs. From 1974 to 1978, more than 25% of the potato germplasm imported by the PQS was found to be infected with one or more viruses (W. J. Kaiser, *unpublished*).

Many viruses naturally infect potatoes wherever they are grown (3,12). Few viruses are transmitted in true potato seed (3,13), whereas most, if not all, are transmitted in vegetatively propagated tubers (3,12). Among the several viruses reported to infect potatoes in Kenya (1,2,20), potato leaf roll virus (PLRV) is probably the most important (2), and alfalfa mosaic virus (AMV) (15) and tomato black ring virus (TBRV) (14) are potentially important. Thus far, the latter two viruses have been limited in distribution to particular areas and certain potato cultivars.

Studies were initiated at the Muguga PQS to develop techniques for producing potato lines free from known viruses (virus-tested). The production of such lines would prevent the destruction of potentially valuable potato germplasm and allow healthy potato material to be available for the Government of Kenya's breeding, disease resistance, and seed certification programs. One of the procedures studied was thermotherapy of virus-infected tubers. In 1949, Kassanis (16) demonstrated the efficacy of heat therapy in freeing potato tubers of PLRV. This study describes the use of treatment with hot air to free tubers of three viruses, AMV, TBRV, and PLRV.

MATERIALS AND METHODS

Source of viruses. All heat-treatment experiments were carried out at the PQS, Muguga, Kenya, with both imported and local potato cultivars that were known by indexing to be virus-infected. These included *S. tuberosum* 'Anett,' 'Cosima,' 'Désirée,' 'Kenya Akiba,' 'Kenya Baraka,' and 'Roslin Eburu (B 53),' and clone 7111/44. Hybrid line A6 (*Solanum demissum* Lindl. × *S. tuberosum* 'Aquila') also was used. Each potato cultivar was indexed by one or more of the following assay methods: host range, physical property, serology, electron microscopy, or vector-transmission studies. Viruses identified were AMV (alfalfa and potato strains), potato virus Y (PVY) (common and veinal necrosis strains), PLRV, and TBRV. One test was conducted with cultivar Anett doubly infected with PLRV and TBRV. Virus isolates were maintained in dormant tubers at 6–8 C, or in potato plants.

All potato tubers, except some from two PLRV-infected cultivars, were harvested from virus-infected mother plants grown in insect-protected greenhouses at the Muguga PQS. Some tubers from the PLRV-infected cvs. Kenya Baraka and Roslin Eburu were increased outdoors in an isolated area at Muguga.

Hot-air treatment of tubers. Dormant (unsprouted) tubers with weights ranging from 0.5 to 25.0 g were kept in paper bags in a walk-in growth chamber (3 m × 3 m × 3 m). Air temperature in the chamber was maintained at 37 C ± 1 C with an Xpelair 3-kw fan heater, and relative humidity was kept at or above 75% by filling large shallow trays with water and by wetting the floor with tap water several times a day. Temperature and relative humidity were recorded continuously with a hygrothermograph. Nonheated control tubers remained at room temperature in paper bags until

planted.

After heat treatment, tubers were placed in a closed container and were treated for 36–48 hr with a Rindite solution (a mixture of ethylene chlorohydrin, ethylene dichloride, and carbon tetrachloride [7:3:1, v/v]) at the rate of 0.5 ml/kg of tubers to induce sprouting. Tubers were stored at room temperature and, after sprouting, were placed in sterilized sand in 12-cm-diameter plastic pots and incubated in an insect-protected greenhouse to induce rooting. After rooting, tubers were transplanted to pasteurized soil in 15-cm-diameter plastic pots. Plants were sprayed periodically with different pesticides to control insects and mites. Greenhouse temperatures ranged from 15–25 C.

Hot-water treatment of tubers. Dormant tubers from virus-infected plants were treated for various times at 50 or 52.5 C in a circulating water bath. Tubers were placed in a wire-screen cage that was submerged in the hot water. After hot-water treatment, tubers were dried at room temperature for 24 hr before treatment with Rindite. Sprouted tubers were transplanted and maintained as described for tubers that received hot-air treatments.

Virus detection. All heat-treated tubers and untreated controls that sprouted were indexed for virus on the following test plants: AMV, *Chenopodium quinoa* Willd. and *Phaseolus vulgaris* L. 'Black Turtle Soup'; PVY, *Nicotiana clevelandii* A. Gray and *N. tabacum* L. 'Samsun'; TBRV, *C. quinoa* and *N. clevelandii;* and PLRV, *Physalis floridana* Rydb. (by aphid transmission). Mechanical indexing was done by triturating foliar tissues in 0.06 M K_2HPO_4 and applying the sap to leaves of young test plants dusted with 0.22-μm (600-mesh) carborundum. Those found to be free of AMV, PLRV, or TBRV were retested for virus infection several times during the first season of growth. Tubers from selected A6 and Anett plants that indexed negative for AMV and TBRV, respectively, were resprouted and reindexed several times during the second growing season. These latter plants also were checked by agar double-diffusion tests (14,15).

The aphid vector used for PLRV was *Myzus persicae* (Sulz.). Virus-free aphid colonies were reared on Chinese cabbage (*Brassica pekinensis* [Lour.] Rupr.). All transmission studies with PLRV were done in the laboratory. A leaf from each potato plant to be tested for PLRV was removed with a sterile scalpel, and its petiole was immersed in 75–90 ml of tap water in a 100-ml beaker. Thirty to 50 nonviruliferous apterous adults and nymphs were allowed a 72-hr acquisition feeding period on each excised leaf. Aphids then were transferred in groups of 10–15 to each of four 10- to 15-day-old seedlings of *P. floridana* for a 72-hr infection feeding interval and were confined to test plants by screened cages. Symptoms of PLRV were recorded in 2–4 wk. Known healthy and PLRV-infected potato plants and healthy *P. floridana* seedlings were included as controls in each transmission test.

TABLE 1. Inactivation of two strains of alfalfa mosaic virus (AMV) in potato tubers maintained at 37 C for various time intervals[a]

Cultivar	Source of AMV	Treatment period (wk)	Survival of tubers	Surviving tubers	
				Infected (%)	Healthy (%)
A6	Alfalfa	0	35/35[b]	86	14
		10	68/85	0	100
A6	Alfalfa	0	43/48	84	16
		2	25/30	68	32
		4	22/30	5	95
		6	16/30	0	100
		8	12/30	0	100
A6	Alfalfa	0	80/80	100	0
		2	23/28	100	0
		3	20/28	70	30
		4	24/28	29	71
		5	24/28	0	100
A6	Potato	0	117/128	100	0
		2	123/128	100	0
		3	95/128	65	35
		4	118/128	19	81
		5	113/128	0	100
		6	112/128	0	100
Kenya Akiba	Potato	0	25/25	100	0
		2	16/25	100	0
		3	13/25	23	77
		4	12/25	0	100
		5	12/25	0	100
		6	15/25	0	100

[a] Weight of unsprouted tubers at the beginning of each experiment ranged from 2 to 10 g for A6 and 0.5 to 4 g for Kenya Akiba.
[b] Number of tubers surviving treatment divided by total number treated.

RESULTS

Inactivation of AMV in tubers by hot-air treatment. Treatment with hot air at 37 C eliminated AMV from 100% of the surviving tubers in potato cultivars Kenya Akiba and A6 after 5-6 wk (Table 1). The curative effect of heat treatment was noticeable after 3 wk, when 30–77% of the tubers were free of virus (Table 1). In two of three heat-treatment experiments with cultivar A6 infected with the AMV isolate from alfalfa, 84 and 86% of the control tubers were found to be infected, and in the third experiment, all of the A6 control tubers were infected. Survival of heat-treated tubers at 5 wk ranged from 48–88% and was lowest in cultivar Kenya Akiba. One factor contributing to poor survival after treatment of the Kenya Akiba tubers may have been their small size.

Isolates of AMV from both alfalfa and potato induced yellow mosaic (calico) symptoms in both potato cultivars, while the foliage of heat-treated plants freed of virus was dark green. The virus could not be detected in dark-green plants by repeated indexing to indicator plants or by serology, whereas diseased plants with calico symptoms indexed positive with both techniques. No virus could be detected by inoculation of test plants or by serology when first-generation tubers from heat-treated, apparently virus-free A6 plants were replanted. Symptoms characteristic of AMV infection were never observed during the development of these plants.

Inactivation of TBRV in tubers by hot-air treatment. In the first experiment, six of the original 10 Anett tubers that survived heat treatment for 10 wk at 37 C were free of TBRV (Table 2). All 42 of the control Anett tubers were infected with TBRV. In the second experiment, TBRV was eliminated from 93% of the tubers after 4 wk of treatment and all tubers were free of this virus after 6 wk of treatment. Survival of tubers at 4 and 6 wk was 93 and 87%, respectively.

All tubers from TBRV-infected controls produced symptomless plants, but the virus could be detected by mechanical inoculation of sap to susceptible indicator hosts, particularly *C. quinoa* and *N. clevelandii.* First-generation tubers were collected and sprouted from 6–10 plants, the tubers of which had originally been heat-treated for 10 wk (exp. 1) and 6 wk (exp. 2). No TBRV could be detected, either by mechanical inoculation to test plants or by serological means, in any of these plants.

Inactivation of PLRV in tubers by hot-air treatment. Potato leaf roll virus was eliminated from tubers of four potato cultivars incubated at 37 C for 3–4 wk (Table 3). Tubers of Cosima and

TABLE 2. Inactivation of tomato black ring virus in infected Anett potato tubers maintained at 37 C for various time intervals[a]

Experiment	Treatment period (wk)	Survival of tubers	Surviving tubers	
			Infected (%)	Healthy (%)
1	0	42/42[b]	100	0
	10	6/10	0	100
2	0	30/30	100	0
	2	30/30	100	0
	4	30/30	100	0
	6	26/30	0	100
	8	22/30	0	100
	10	10/30	0	100

[a] Weight of unsprouted tubers at the start of both experiments averaged 10 g.
[b] Number of tubers surviving treatment divided by total number treated.

Kenya Baraka were free of PLRV after 3 wk of hot-air treatment, whereas 4 wk of treatment was required to eliminate PLRV from all tubers of Désirée and Roslin Eburu. Survival of tubers ranged from 56–100% after 4 wk of treatment at 37 C.

Greenhouse-grown plants of the four cultivars varied considerably in symptoms of infection with PLRV. Symptoms were most severe in Kenya Baraka and mildest in Désirée. Since visual observations were not reliable for detecting PLRV-infected plants under greenhouse conditions, each potato plant was indexed by aphid transmission to *P. floridana*. Aphid transmission tests were effective for detecting PLRV in symptomless plants. By this technique, no PLRV was detected in first-generation tubers from plants arising from heat-treated tubers (37 C for 3–4 wk). The virus was also eliminated from smaller batches (2–20 tubers) of over 50 imported and Kenyan breeding lines and cultivars by hot-air treatments at 37 C for 4 wk. In tubers that had sprouted before being subjected to 4 wk of hot-air therapy, survival was poor (usually <10%).

Failure of hot-air treatment to inactivate PVY in tubers. Heat treatment at 37 C for periods up to 10 wk did not eliminate either of two PVY strains from tubers of cultivar Anett or clone 7111/44 (Table 4). Survival of tubers after 10 wk of heat treatment ranged from 33–40%.

All heat-treated tubers that sprouted contained PVY and produced plants that exhibited typical symptoms of stunting, mosaic, and leaf deformation and curling like those of unheated controls.

Simultaneous eradication of PLRV and TBRV from heat-treated Anett tubers. Thirty plants, the tubers of which were collected from six Anett potato plants that were infected simultaneously with PLRV and TBRV indexed free of both viruses after heat treatment of the tubers for 6 wk at 37 C. All 30 plants from nonheated control tubers tested positive for both viruses. Dually infected plants had symptoms of PLRV; TBRV infection was symptomless in these plants.

Failure of hot-water treatment to free tubers of AMV, PLRV, and TBRV. Hot-water treatment at 50 C for 25–180 min or 52.5 C for 15–90 min did not eliminate AMV, PLRV, or TBRV as determined by indexing plants from more than 100 dormant tubers of cultivar A6, Kenya Akiba, Cosima, Désirée, Kenya Baraka, Roslin Eburu, or Anett. One plant from a tuber treated for 160 min was free of AMV, but this could have been a natural escape. Survival of tubers of all potato cultivars treated with hot water at 50 C for periods of up to 120 min was >75%. Survival of tubers treated for more than 120 min decreased to <50%.

DISCUSSION

This study has demonstrated the effectiveness of hot-air, but not hot-water, treatments for eliminating at least three viruses from small batches of tubers of several imported and Kenyan potato cultivars. Thermotherapy has enabled the Muguga PQS to produce virus-tested mother plants of several potato cultivars from which tubers and rooted cuttings are obtained for distribution to various potato improvement programs in East Africa.

Since its introduction in 1949 (16), heat therapy has been extensively used to rid tubers of PLRV (5,9,10,17,19,23–25,28,30). However, heat treatment has not been successful in freeing potato tubers from other viruses, including potato acuba mosaic virus (25), potato virus A (25), potato virus S (26), potato virus X (4,5,17,25,29), PVY (17,25,29), tobacco rattle virus (25) and the viroid-induced potato spindle tuber disease (5). In a bulletin published in 1977 by the International Potato Center, Lima, Peru, on the major diseases and nematodes of potato, PLRV was the only virus listed as being eliminated from potato tubers by heat treatment (12). The present study has expanded the list of viruses that can thus be eliminated from potato tubers by thermotherapy to include AMV and TBRV.

Recently, several new potato viruses have been described from the potato-growing areas of the Andes region (6,7,8,12,13,27). Most of these viruses are comprised of spherical particles. As yet, there are no reports on the effects of heat treatment in eradicating these newly described viruses from potato tubers. It is possible that some of the new potato viruses will be eliminated from tubers by hot-air treatment, as were AMV, PLRV, and TBRV in the present study. However, heat therapy did not free potato tubers of rod-shaped viruses in this study and in others (4,5,17,25,26,29).

The reported control of PLRV in tubers of one potato cultivar by hot-water treatment at 50–55 C for 17–20 min (19) would considerably reduce the time and space required to free tubers of virus infection. My results and those of others (4,17,24) did not agree with those of Nagaich and Upreti (19,30). Several factors could have contributed to these discrepancies; eg, different virus-

TABLE 3. Inactivation of potato leaf roll virus in infected tubers of four potato cultivars maintained at 37 C for various time intervals[a]

Cultivar	Treatment period (wk)	Survival of tubers	Surviving tubers	
			Infected (%)	Healthy (%)
Roslin Eburu	0	14/14[b]	100	0
	1	14/14	100	0
	2	12/14	100	0
	3	14/14	29	71
	4	14/14	0	100
	5	14/14	0	100
	6	12/14	0	100
Désirée	0	14/14	100	0
	1	13/14	100	0
	2	12/14	100	0
	3	12/14	8	92
	4	13/14	0	100
	5	14/14	0	100
	6	9/14	0	100
Cosima	0	16/16	100	0
	1	16/16	100	0
	2	16/16	100	0
	3	16/16	0	100
	4	9/16	0	100
	5	14/16	0	100
	6	12/16	0	100
Kenya Baraka	0	16/16	100	0
	1	13/16	100	0
	2	14/16	100	0
	3	11/16	0	100
	4	16/16	0	100
	5	11/16	0	100
	6	12/16	0	100

[a] The weight of unsprouted tubers at the beginning of each experiment ranged from 1 to 20 g.
[b] Number of tubers surviving treatment divided by total number treated.

TABLE 4. Lack of inactivation of two strains of potato virus Y in virus-infected tubers of potato clone 7111/44 and cultivar Anett maintained at 37 C for various time intervals[a]

Clone or cultivar	Strain of virus	Treatment period (wk)	Survival of tubers	Surviving tubers	
				Infected (%)	Healthy (%)
7111/44	Veinal necrosis	0	30/30[b]	100	0
		6	24/30	100	0
		7	27/30	100	0
		8	20/30	100	0
		9	20/30	100	0
		10	12/30	100	0
Anett	Common	0	14/14	100	0
		2	7/9	100	0
		4	3/9	100	0
		6	4/9	100	0
		8	2/9	100	0
		10	3/9	100	0

[a] Average weight of unsprouted tubers at the start of each experiment was 5 g for clone 7111/44 and 10 g for cultivar Anett.
[b] Number of tubers surviving treatment divided by total number treated.

detection methods, potato cultivars, or strains of the virus. Upreti and Nagaich (19,30) determined infection with PLRV by visible symptoms or by the phloroglucinol staining method, both of which are generally much less sensitive and reliable than aphid transmission to *P. floridana,* which was used in the present study (3,11). Potato cultivars frequently differ in the severity of symptoms on plants infected with a given strain of PLRV (3,22). Such differences could complicate detection of virus infection in some hosts by visual means, histological methods, or both. Several strains of PLRV have been described (3,22), and some appear to vary in heat tolerance (19). The strain(s) of PLRV infecting tubers of the four Kenyan cultivars may have been more heat tolerant than those studied by Upreti and Nagaich (30). Hot-water treatment to control certain tuber-borne potato viruses appears to have potential, but it requires additional investigation.

Heat therapy and meristem-tip culture have been used extensively, singly and in combination, to free potatoes of several viruses (11,18,21,23). Although heat treatment requires less skill and less-specialized laboratory facilities than tissue culture, it has, until now, only been effective in freeing potato tubers of PLRV. This is the first report of the successful use of thermotherapy on the African continent to free potato tubers of virus infection. The thermotherapy procedures described herein may be of interest and use to research institutions and plant quarantine stations where there are fears of introducing virus-induced diseases in imported potato germplasm source material, and to those that distribute potato germplasm for research and commercial purposes.

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