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OCCURRENCE OF TOMATO BLACK RING VIRUS IN POTATO CULTIVAR ANETT IN KENYA

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ABSTRACT

A virus isolated from diseased Anett potatoes imported into Kenya was identified on the basis of host range, particle morphology, physical properties, and serology as tomato black ring virus (TBRV). The Anett isolate was serologically related to the beet ringspot virus group of TBRV. It did not react with antiserum to the type (tomato) strain of TBRV or to antisera to tobacco ringspot, tomato ringspot, or raspberry ringspot viruses. The host range of the Anett isolate was extensive and the virus was seedborne in several artificially inoculated food crops and weed hosts. The virus was not transmitted by aphids or the nematode Longidorus laevicapitatus which occurs in soils of the potato-growing regions of Kenya. This is the first published report of TBRV on the African Continent. The importance of strict observance of quarantine in importations of potatoes is thus underlined.

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Additional key words: nepovirus, seed transmission, Solanum tuberosum.

Potatoes (Solanum tuberosum) have been cultivated in the highlands of Kenya for over 50 years where they have become an important food and cash crop (1, 2). In the last 10 years, tubers of numerous potato lines have been introduced into Kenya for testing. The primary aim of these importations has been to increase potato production through breeding and selection of varieties resistant to important diseases, particularly late blight, bacterial wilt, and viruses.

The movement of potato germplasm into Kenya is governed by a set of plant import regulations known as the Plant Protection (Importation) Order. All seed tubers entering Kenya from countries other than specified sources in the United Kingdom, Ireland, and the Netherlands should be processed through the Plant Quarantine Station, Muguga, to exclude potential pests and pathogens of potato from Kenya.

In October 1976, a stunted Anett potato plant with shortened internodes was delivered to the Plant Quarantine Station, Muguga. The diseased plant collected from the Grasslands Research Station, Marindas, Kenya was infected with tomato black ring virus (TBRV), which is prevalent in Europe (7). All of the TBRV-infected Anett potato plants found in Kenya appear to have originated from one consignment of Anett tubers that was imported from West Germany in the mid-1960's for research purposes.

The present study was undertaken to assess the relationship of the Anett isolate of TBRV to other nepoviruses and to investigate the biological and physiological properties of the virus.

MATERIALS AND METHODS

An isolate of TBRV from Anett potato collected at the Grasslands Research Station, Marindas, was used in all host range, seed and vector transmission, and serological studies.

Isolates of TBRV were maintained in Nicotiana tabacum cv. Samsun or N. clevelandii. Test plants were grown in steam-sterilized soil in insect-protected glasshouses (temp 15-30° C).

Infected leaves of N. clevelandii were triturated in 1% K_2HPO_4 or tap water and the sap was applied to carborundum-dusted leaves of test plants. After 4-5 weeks, all plants were back-inoculated at least three times to Chenopodium quinoa and N. clevelandii to establish whether systemic infection had occurred Seeds from TBRV-infected plants were sown in steam-sterilized soil and seedlings were indexed separately on C. quinoa indicator plants.

Physical property tests were made with crude infective sap of N. clevelandii and the assay made on C. quinoa. Dilution-end points were determined by serial dilutions in distilled water.

Vector transmission: Soil samples were collected from field plots at Marindas, Njabini, and Tigoni, Kenya where TBRV-infected Anett potato plants had grown. Nematodes were extracted from soils by wet screening 250-500 g of soil on 0.71-mm and 0.053-mm sieves. The sediment from the 0.053-mm sieve was washed into several sheets of Kleenex facial tissue contained in a beaker. Samples of filtrate were observed for pathogenic nematodes after 2 hr. The remainder of each soil sample was sown with C. quinoa and Cucumis sativus 'National Pickling' bait plants, the roots and foliage of which were indexed separately on C. quinoa and N. clevelandii after 5 weeks. Subsequently, these soils were planted with TBRV-infected Anett potatoes that were harvested after 90 days. The soils were sown on separate occasions with C. quinoa, Beta vulgaris 'Detroit Red', and Anett potato bait plants that were indexed as above for the presence of virus.

Nonviruliferous colonies of Myzus persicae were reared on healthy Chinese cabbage. Aphids were starved for 1-2 hr in clean petri dishes before being given acquisition feeding periods of less than 1 min (duration of a single probe) to 72 hr on TBRV-infected Anett potato leaf petioles. After acquisition feeding, aphids were transferred in groups of 10-20 aphids to healthy N. clevelandii for 72 hr at which time plants were sprayed with an aphicide.

Purification: TBRV was purified by homogenizing 100 g of systemically infected N. clevelandii leaves in M15 (67 mM) phosphate buffer containing 0.001 M ethylenediaminetetraacetic acid and 0.1% thioglycolic acid (pH 7.2). The homogenate was squeezed through nylon cloth and mixed with an equal volume of a 1 : 1 mixture of butanol and chloroform, stirred for 10 min, and clarified by centrifugation at 2,000 g for 10 min and 30,000 g for 20 min. The supernatant was centrifuged at 78,000 g for 120 min, and the small clear pellets resuspended for 60 min in 0.01 M phosphate buffer, pH 7.5. Two further differential centrifugation cycles were made; the final pellet being resuspended in 1.0 ml of the phosphate buffer. Partially purified virus preparations (0.3-0.5 ml) were layered on sucrose density gradients and centrifuged in a Beckman SW 25 Rotor for 180 min at 24,000 rev/min.

Antiserum preparation and serology: Rabbits were injected intravenously with 1 ml of a purified virus preparation, followed 7 days later by an intramuscular injection of 1 ml of virus emulsified with 1 ml of Freund's incomplete adjuvant, and again 14 days later by a second, similar injection. Rabbits were bled 21-28 days after the final injection. Ouchterlony tests were made at room temperature in agar-gel plates. Serological tests also were made in precipitation tubes incubated at 37° C in a water bath. Final records of precipitin reactions were made after 3 or 4 hr. Purified preparations of the Anett isolate were tested against antisera of the following nepoviruses: a TBRV strain from beet (beet ringspot strain: TBRV-B), a TBRV strain from lettuce (lettuce ringspot strain: TBRV-L), the TBRV type strain from tomato (TBRV-T), tomato ringspot virus (TomRSV), tobacco ringspot virus (TRSV), and raspberry ringspot virus (RRSV).

Analytical centrifugation: Sedimentation determinations were made in a Beckman Spinco Model E analytical centrifuge with Schlieren optics.

Electron microscopy: Purified virus preparations were mixed with an equal volume of 2% sodium phosphotungstate, pH 7.0, and sprayed onto 3-mm carbon-coated grids. All specimens were examined in a Siemens Elmiskop 1A electron microscope.

RESULTS

Distribution of TBRV: TBRV was isolated from Anett potatoes cultivated in the Kenya highlands at Marindas, Njabini, and Tigoni. The virus was not isolated from crops and weeds growing in close proximity to the TBRV-infected potatoes. Symptomatology was not reliable for detecting TBRV-infected Anett potatoes in the field because infected potatoes frequently were symptomless. From host range studies, the Anett plant from Marindas appeared to be infected only with TBRV. Tubers from the infected plant gave rise to symptomless plants, although all still were infected with TBRV. Repeated attempts to infect mechanically healthy Anett potato plants with TBRV failed.

Host range studies: Of 63 plant species tested, the Anett isolate infected 48 species in 1[illegible] families. Susceptibility to TBRV was demonstrated for the first time (indicated by a +) [illegible]7 species. The Anett virus induced symptoms in diagnostic plant species (Figs. 1-7) sim[illegible] to those described by Murant (7). The virus produced local lesions without systemic sp[illegible] in Cyamopsis tetragonoloba+ (Leguminosae) and Solanum tuberosum 'Sebago' (Solanaceae). Systemic symptoms, with (indicated by an asterisk) or without local lesions, occurred in Gomphrena globosa* (Fig. 7) (Amaranthaceae); Beta vulgaris 'Detroit Red'*, Chenopodium amaranticolor* (Fig. 1), C. fasciculosum var. muraliforme*+, C. murale*, C. quinoa* (Fig. 2), and Spinacia oleracea 'Bloomsdale Long Standing'* (Chenopodiaceae); Helianthus annuus, Tagetes minuta+ (Compositae); Cucumis sativus 'National Pickling', Cucurbita maxima 'White Bush'* (Cucurbitaceae); Ocimum basilicum* (Labiatae); Cajanus cajan 'T-21'+; Cicer arietinum+, Glycine max 'HLS 541'*, Lathyrus odoratus*, Phaseolus vulgaris 'Black Turtle Soup'*, Pisum sativum 'Dark Skin Perfection', Trigonella foenum-graecum*+, Vicia faba 'Aquadulce'*, Vigna radiata*+, V. unguiculata 'California No. 5'* (Fig. 5), Voandzeia subterranea+ (Leguminosae); Gossypium hirsutum 'Ac 44'*+, Hibiscus canabinus*+ (Malvaceae); Datura stramonium* (Fig. 6), Lycopersicon esculentum 'Rutgers'*, Nicotiana clevelandii* (Fig. 3), N. debneyi*, N. glutinosa*, N. tabacum 'Samsun'*, (Fig. 4), Nicandra physalodes*, Petunia hybrida 'Single Mixed'*, Physalis floridana, Solanum nigrum* (Solanaceae). The virus was systemic, but remained symptomless in Amaranthus graecizans+ (Amaranthaceae); Chrysanthemum cinerariifolium+, Galinsoga parviflora*, Lactuca sativa 'Black Seeded Simpson', Zinnia elegans 'Dahlia-flowered Mixed' (Compositae); Manihot esculenta 'Kibandameno'+ (Euphorbiaceae); Arachis hypogaea 'Natal Common'+ (Leguminosae); Portulaca oleracea (Portulacaceae); Rubus idaeus 'Malling Jewel' (Rosaceae); Solanum melongena 'Ghalami'+ (Solanaceae); Daucus carota+ (Umbelliferae). No symptoms developed on, and no virus was recovered from Chrysanthemum morifolium, Dahlia variabilis (Compositae); Brassica oleracea var. capitata 'Prize Drumhead', B. pekinensis (Cruciferae); Citrullus lanatus 'Congo' (Cucurbitaceae); Ricinis communis (Euphorbiaceae); Sorghum bicolor 'Serena', Zea mays 'H 512' (Gramineae); Medicago sativa 'Hairy Peruvian', Phaseolus lunatus 'Jackson Wonder' (Leguminosae); Allium cepa 'Creole' (Liliaceae); Capsicum frutescens 'Long Red Cayenne', Datura metel, Solanum demissum x S. tuberosum 'A-6', S. tuberosum 'Anett' (Solanaceae); Petroselinum crispum (Umbelliferae).

Seed transmission: TBRV was seedborne but was usually symptomless in 0.7 to 97% of the seedlings of 15 species in 6 families. A majority of these are new records of seedborne hosts of TBRV (indicated by a +). Seed transmission was detected in Amaranthus graecizans+ (4 seedlings infected out of 90 tested), Cajanus cajan+ (1/4), Chenopodium fasciculosum var. muraliforme+ (1/3), C. quinoa+ (3/4), Datura stramonium+ (5/100), Glycine max 'HLS 541' (65/67), Gomphrena globosa+ (5/33), Helianthus annuus+ (4/77), Nicandra physalodes+ (12/60), Nicotiana debneyi+ (3/100), Phaseolus vulgaris 'Black Turtle Soup'+ (1/144), Portulaca oleracea+ (5/50), Vigna radiata+ (27/40), V. unguiculata (7/8), and Zinnia elegans+ (1/4). No seed transmission was detected in Chenopodium murale (0/65), Cicer arietinum (0/21), Galinsoga parviflora (0/100), Hibiscus canabinus (0/50), Lycopersicon esculentum 'Rutgers' (0/113), N. clevelandii (0/154), N. tabacum 'Samsun' (0/60), N. tabacum 'White Burley' (0/100), and Pisum sativum 'Dark Skin Perfection' (0/18).

Physical property tests: The in vitro properties of the Anett isolate of TBRV were as follows: Thermal inactivation, some infection after heating 10 min at 59°C, but none at 60° C; dilution end-point, infection at 10^{-5}, none at 10^{-6}; longevity in vitro at room temperature, some infection at 19 days, but none at 20 days.

Vector transmission: Several pathogenic nematodes were found in soil samples collected at Marindas, Njabini, and Tigoni. These were Helicotylenchus, Hoplolaimus, Longidorus, Meloidogyne, Pratylenchus, Radopholus, and Xiphinema spp. All Longidorus appeared to belong to one species, L. laevicapitatus, which is widely distributed in tropical regions on various crops. Most soil samples contained specimens of this Longidorus sp. TBRV was not isolated from the roots or foliage of C. quinoa, B. vulgaris, C. sativus, or S. tuberosum bait seedlings that were sown in the soil samples, either before or after planting with TBRV-infected Anett potatoes. Myzus persicae did not transmit TBRV from Anett potato to N. clevelandii.

Electron microscopy: Purified preparations of TBRV contained numerous isometric particles about 30 nm in diameter. Characteristic of nepovirus particles, some were completely penetrated by the negative stain, some partially, and some not (Fig. 9).

Analytical centrifugation: Particles sedimented as three components with sedimentation coefficients (S_{20W}) of 50 (top component), 98 (middle), and 120 (bottom).

Sucrose density gradient centrifugation: Purified preparations usually separated into three components in sucrose gradients (Fig. 8). Occasionally only two zones, middle (M) and bottom (B), formed. The top component was not infectious, but both the M and B components were.

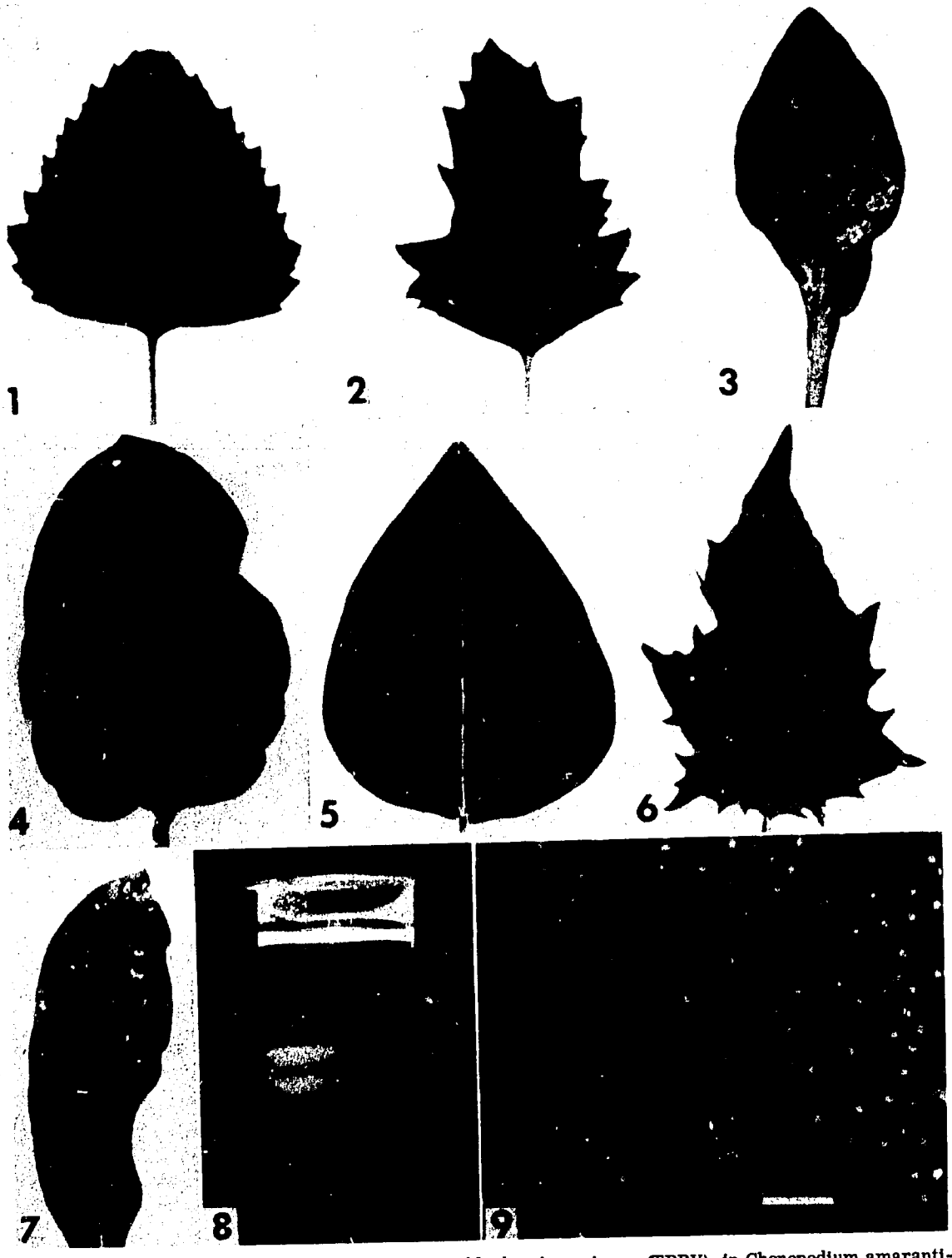

FIGURE 1. Local necrotic lesions of tomato black ring virus (TBRV) in Chenopodium amaranticolor. FIGURE 2. Local necrotic lesions in C. quinoa. FIGURE 3. Local ringspot lesions in Nicotiana clevelandii. FIGURE 4. Systemic line patterns in N. tabacum 'Samsun'. FIGURE 5. Local necrotic lesions in Vigna unguiculata. FIGURE 6. Systemic chlorotic blotches in Datura stramonium. FIGURE 7. Local necrotic lesions in Gomphrena globosa. FIGURE 8. Top (t), middle (m), and bottom (b) component separation in sucrose density gradients (see text). FIGURE 9. Particles of TBRV strained in 2% potassium phosphotungstate. Bar represents 100 nm.

The 260/280 absorbance ratio was 1.75-1.85 for unfractionated preparations.

Serology: Antiserum to the Anett isolate had a titer, against homologous TBRV, of 1/16,384 in tube precipitin tests and 1/512 in agar-gel tests. In standard agar-gel tests, the Anett isolate reacted strongly with an antiserum prepared against TBRV-B to a reciprocal titer of 512 (titer of the antiserum against its homologous virus was 1,024), only weakly (1/4) against antisera prepared against TBRV-L (2,048), and not at all with TBRV-T (1,024), or with the antisera prepared against TomRSV (2,048), TRSV (16,384), or RRSV (256).

DISCUSSION

On the basis of particle morphology and sedimentation coefficients, serology, physical properties, and host range and reactions, we have identified the Anett potato virus as TBRV: all its characters are similar to, if not identical with, those described for TBRV (7). Our tests clearly place the Anett virus into one of the two described TBRV groups (7), namely that containing the beet ringspot and potato pseudoaucuba strains.

This is the first published report of TBRV on the African continent. Although the experimental host range of TBRV, including the Anett isolate, is extensive (3,5,7,8,9,10,11), the virus was isolated only from naturally infected Anett potatoes in Kenya (and not other potato varieties). It seems likely, therefore, that TBRV was introduced into Kenya in virus-infected Anett potato tubers. This illustrates the ease with which new and potentially dangerous pathogens, like TBRV, can be introduced into new areas when special precautions are not taken.

TBRV is potentially of great importance to local agriculture. In glasshouse inoculation studies, the Anett isolate infected many food and cash crops grown in Kenya. The Anett isolate was transmitted in seed of several artificially inoculated weed species that commonly grow in association with potatoes, and several of these species are new records of seedborne hosts of TBRV. TBRV is seedborne in at least 24 species in 15 families (6,7) and in nature, seed transmission is important in dissemination and survival of the virus (4,6,7).

We were unable to demonstrate nematode transmission of the Anett isolate to C. quinoa, cucumber, sugarbeet, and potato bait seedlings in L. laevicapitatus-infested field soils, in which TBRV-infected Anett plants had grown. Two Longidorus species, L. attenuatus and L. elongatus, are vectors of TBRV in Europe (4,7,11). Different strains of TBRV are transmitted by these two species (4), neither of which has been recorded in Kenya. The nematode vector-virus strain specificity may account for the failure of L. laevicapitatus to transmit TBRV, but additional testing is required to establish beyond doubt that L. laevicapitatus is not a vector of TBRV under Kenya conditions.

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