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Serological relationships and purification of bud necrosis virus, a tospovirus occurring in peanut (*Arachis hypogaea* L.) in India*

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Summary

A procedure for the purification of a tospovirus which causes bud necrosis disease (BND) of peanut in India is described. The virus contained three polypeptides of 78 kDa, 54 kDa and 31 kDa. In two ELISA procedures the virus failed to react with antisera to tomato spotted wilt virus (TSWV) obtained from different sources and with an antiserum to impatiens necrotic spot virus (INSV). Additionally, in reciprocal tests TSWV and INSV antigens failed to react with antiserum to the virus infecting peanut in India.

In electro-blot immunoassay 54 kDa and 31 kDa polypeptides of the virus reacted with the homologous antiserum. None of the heterologous antisera reacted with any of the three viral polypeptides. On the basis of serological differences the virus that causes BND in India is distinct and therefore has been named bud necrosis virus (BNV). This serotype appears to be restricted to Asia.

Key words: Bud necrosis virus, tomato spotted wilt virus, impatiens necrotic spot virus, ELISA, electro-blot immunoassay, serotypes, tospoviruses

Introduction

The occurrence of bud necrosis disease (BND) on peanut (= groundnut, *Arachis hypogaea* L.) was first reported from India by Reddy, Reddy & Rao (1968). It was subsequently shown that BND was caused by a virus which resembled tomato spotted wilt virus (TSWV) (Ghanekar *et al.*, 1979; Reddy *et al.*, 1991). Since the early 1960s BND has been a major constraint to peanut production in several parts of India (Reddy *et al.*, 1991).

Infectivity of BND-infected leaf extracts is dependent upon several factors, the most important of which are the addition of suitable antioxidants to the inoculum and the age of the leaf tissues used. This has hampered earlier attempts to identify the causal virus on the basis of host range and reaction (Reddy, 1988). Also, antisera for TSWV from other countries did not react with the virus obtained from BND-infected plants (Anon., 1989; Sreenivasulu *et al.*, 1991). It was therefore essential to produce antisera against the virus causing BND in India.

Initially, several available methods were tried to prepare virus samples largely devoid of host material. Such preparations are required for production of good quality polyclonal

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antisera and subsequent development of methods for virus detection and also determination of serological relationships. We report in this paper on purification procedures and the serological relationships of the virus that causes BND of peanut in India

Materials and Methods

Virus isolate

Extracts from virus-infected peanut samples, prepared in 0.05 M potassium phosphate buffer, pH 7.2, containing 2 g/litre thioglycerol were inoculated onto cowpea (*Vigna unguiculata*, cv. C 152). Virus obtained from a single lesion was reinoculated onto cowpea and after three successive single lesion transfers in cowpea was maintained in peanut (cv. TMV-2) by sap inoculation.

Virus purification

Systemically infected young quadrifoliates of peanut showing primary symptoms, frozen at −70°C, were used. Leaflets were homogenised (4 ml/g tissue) in chilled 0.1 M potassium phosphate buffer, pH 7.5, containing 0.01 M sodium sulphite (0.1 M PPBS). The extract was squeezed through two thicknesses of cheese-cloth and centrifuged at 4000 *g* for 10 min. To the supernatant, sodium chloride and polyethyleneglycol (PEG, MW 8000, Fisher Scientific Co.) were added at 11.7 g/litre and 40 g/litre respectively, and after dissolving, the mixture was held at 4°C. After 90 min to 2 h, the precipitate was collected by centrifuging at 8000 *g* for 15 min and suspended in 0.01 M PPBS (0.3 ml/g initial tissue). After centrifugation at 4000 *g* for 15 min, the supernatant was layered onto 200–600 g/litre preformed sucrose gradients (prepared by layering 8 ml of each of 200 and 300 g/litre, and 12 ml of 600 g/litre sucrose in 0.01 M PPBS, and held overnight at 4°C). Gradients were centrifuged at 23 000 rpm for 45 min in a Beckman SW 28 rotor. A diffuse light-scattering zone, at 2.6 to 3.1 cm from the bottom, was collected. Pellets found in gradient tubers were also suspended in 0.01 M PPBS at a rate of 2 ml for each pellet. Sucrose zones and resuspended pellets were mixed, stirred at 4°C for 1 h, layered onto 300–600 g/litre preformed linear sucrose gradients (prepared by layering 7 ml of each of 300, 400, 500 and 600 g/litre sucrose in 0.01 M PPBS and leaving overnight at 4°C) and centrifuged at 23 000 rpm for 2.5 h in the SW 28 rotor. A light-scattering zone between 2.5 to 3.1 cm from the bottom of the tube was removed, diluted in 0.01 M PPBS, and processed through another cycle of gradient centrifugation as described above. A single light-scattering zone between 2.7 to 3.0 cm from the bottom of the tube was collected, diluted in 0.01 M PPBS, and the virus was pelleted at 35 000 rpm for 2 h in a Beckman R 50 rotor.

For the removal of proteins, presumed to be of host origin, virus preparations from the 200–600 g/litre sucrose gradients were treated with 5 ml/litre Nonidet P-40 (de Avila *et al.*, 1990) in 0.01 M PPBS, gently stirred for 30 min at 4°C, and centrifuged (R 50 rotor; 2 h; 35 000 rpm).

Antiserum production

Purified virus (from 100 g peanut leaves, treated with Nonidet P40 suspended in 1.0 ml of 0.02 M potassium phosphate buffer, pH 7.0, containing 8.5 g/litre sodium chloride), emulsified with an equal volume of Freunds' incomplete adjuvant, was injected intramuscularly into a New Zealand White inbred rabbit at weekly intervals for 6 wk. The rabbit was bled 10 days after the final injection and thereafter at weekly intervals.