Pak. J. Bot., 34(3): 315-322, 2002.

# MOVEMENT OF BEET CURLY TOP VIRUS OUT OF PHLOEM CELLS IN BEAN PLANTS CO-INFECTED WITH TOBACCO MOSAIC VIRUS

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**Abstract**

Pinto bean (*Phaseolus vulgaris* L.), cultivar ouray, was inoculated with beet curly top virus (BCTV) at the crook neck seedling stage using beet leafhoppers (*Circulifer tenellus* Baker). After 7-10 days, primary leaves of each plant were rub-inoculated with one of two isolates of tobacco mosaic virus (TMV). These plants were routinely infected by both viruses. Infection with BCTV did not affect TMV movement or symptoms. TMV remained confined in the local lesions and could not be detected in systemic tissue by enzyme-linked immunosorbent assay (ELISA), tissue blotting, or by local lesion assay. In contrast, infection with TMV did affect BCTV infection. Severe BCTV symptom developed in the growing points of bean plants that were also infected with TMV while relatively mild BCTV symptoms developed on plants infected with BCTV only. Concentration of BCTV in plants infected by both viruses was 8-10 fold higher than in plants infected with BCTV only. Using tissue-blotting ELISA, it was demonstrated that BCTV escaped the phloem and invaded the parenchyma cells of both shoots and roots in plants infected by both BCTV and TMV. However, BCTV remained confined to phloem tissue in plants infected only with BCTV. No difference was observed between two TMV isolates used regarding their effectiveness in enhancing the concentration of BCTV in systemic leaf tissue and in roots of co-infected plants. When plants were first inoculated on primary leaves with TMV and later inoculated with BCTV, neither control nor test plants were systemically infected by BCTV.

## Introduction

Beet Curly Top Virus (BCTV), a geminivirus, causes an important disease of beans (*Phaseolus vulgaris* L). It is transmitted by the beet leafhopper, *Circulifer tenellus* Barker (Davies *et al.*, 1987; Thomas & Boll, 1977). The small isometric particles of BCTV (20-24 nm in diameter) occur both singly and as twins (geminate particles), both forms are infectious (Mumford, 1974). Geminivirus genomes are either bipartite, with two circular DNA molecules or monopartite, with only one circular DNA molecule (Davies *et al.*, 1987). Bipartite viruses, like bean golden mosaic virus (BGMV), are white fly-transmitted (Bird *et al.*, 1973) while monopartite geminiviruses, like BCTV, are leafhopper transmitted (Stanley *et al.*, 1986; Thomas & Boll, 1977).

It has been reported that the DNA-2 of bipartite BGMV codes for two putative movement proteins provide cell-to-cell movement (Rogers *et al.*, 1986; Stanley & Gay, 1983; Sunter *et al.*, 1987). Since, BCTV lacks the DNA-2 molecule, and is a phloem-restricted virus, it may not possess cell-to-cell movement function (Stanley *et al.*, 1986).

In a number of studies, viruses that otherwise remain restricted in localized infections or to phloem tissue can be stimulated to move systemically by co-infections with other viruses (Carr & Kim, 1983; Dodds & Hamilton, 1972; Hamilton & Nichols, 1977; Jayasinghe *et al.*, 1989; Taliansky *et al.*, 1982; Taliansky *et al.*, 1982; Thomas & Ahmed, 1991). Presumably, the helper viruses provide movement function lacking in the first virus. Carr & Kim (1983), showed that BGMV, invaded the non-phloem tissue of bean plants in the presence of Tobacco Mosaic Virus (TMV) but remained confined to the phloem tissue in the absence of TMV.

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TMV infects pinto beans but remains confined to local lesions on inoculated primary leaves. BCTV also infects pinto beans and remains restricted to phloem tissue. The objective of this study was to determine the effects of dual infection with TMV and BCTV on systemic movement of these viruses in pinto beans.

## Materials and Methods

**Host plants:** Seeds of pinto bean variety ouray were germinated in sand in clay pots. Young seedlings were then transplanted in the crook neck stage to 10 cm plastic pots, kept in a greenhouse at temperatures between 20-24°C. Liquid fertilizer consisting of 6% nitrogen, 6% phosphorus and 6% potassium diluted 1:200 was continuously added to the irrigation water.

**Insect culture:** Viruliferous leafhoppers (*C. tenellus* Barker) were reared on infected BCTV sugar beet (*Beta vulgaris* L.) cultivar N.B-4 These leafhoppers and sugar beet plants were kept in an isolated insectory at 28-34°C with 16 hours artificial light. The plants were regularly tested using ELISA for the presence of BCTV.

**Virus transmission:** Plants were inoculated with BCTV using leafhoppers (*C. tenellus*). Leafhoppers, 8-10 per plant, were placed in cages on bean seedlings at the crook neck stage for 48 hours. An additional 8-10 new leaf hoppers per plant were introduced again into the inoculation cages and allowed to feed for another 24 hours to reduce the chances of escape from infection. At the end of the transmission period, leafhoppers were killed using nicotine sulfate fumigation.

TMV inoculum was prepared by grinding fresh diseased leaf tissue diluted 1:10 w/v in 50 mM potassium phosphate buffer, pH 7.00, using a mortar and pestle. TMV was rub-inoculated on Carborundum-dusted (600 mesh) primary leaves of each plant with a cheesecloth pad moistened in inoculum. For inoculations, plants were divided into two sets. In the first set, plants were inoculated with BCTV at the crook-neck stage and then with TMV 7-10 days after the development of primary leaves. Control plants were inoculated with only TMV, BCTV or kept non-inoculated. In the second set, primary leaves were first inoculated with TMV and then with BCTV 3-4 days after the development of local TMV lesions. Control plants were inoculated with TMV only, with BCTV only, or kept non-inoculated. The experiment was repeated two times with six test plants in each treatment and four plants in control groups. After the completion of the inoculation process, plants were kept in a greenhouse with supplementary light provided as needed to maintain 16 hour day-length by rotary, low pressure, 1000 watt, sodium lamps (70,000 lug). The temperature was maintained between 20-24°C. Liquid fertilizer consisting of 6% nitrogen, 6% phosphorus and 6% potassium diluted 1:200 was continuously added to the irrigation water.

**Virus isolates:** Two TMV isolates, U-1 and M supplied by Dr. G. I. Mink were used. TMV isolate U-1 was maintained on plants of *N. tabacum* cultivar Xanthi which were incubated in a greenhouse at 20-24°C. The second TMV isolate-M was purified. BCTV isolate used in this study was collected from Prosser, Washington from sugarbeet plants and was maintained on *B. vulgaris* cv. N.B-4 kept in an isolated insectory with leafhoppers at 28-34°C.