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Standardization of a Graft Inoculation Method for the Screening of Mungbean Germplasm against *Mungbean yellow mosaic virus* (MYMV)

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This report described a simple, inexpensive, faster, and effective graft inoculation method for the artificial transmission of *Mungbean yellow mosaic virus* (MYMV). Success of grafting and disease transmission was 100% in this method. Screening of mungbean germplasm using this method will prevent the chance of escape infection, probably as a consequence of non-preference mechanism and loss of vector infectivity. The grafting method described here is applicable to both screenhouse and field trials.

Keywords **:** Germplasm, graft inoculation, mungbean, MYMV, screening, *Vigna radiata.*

Mungbean (*Vigna radiata* (L.) Wilezek) is an important short duration grain legume crop in South Asia and is a rich source of dietary protein for the poor. Resource-poor farmers prefer to cultivate mungbean than other crops because it does not require lots of water or other inputs and it helps restore soil fertility through symbiotic nitrogen fixation (Rachie and Roberts, 1974). Worldwide annual cultivation of mungbean ranges from about 2.5 million tons to 5 million hectares.

Economic yields of pulses are low due to various biotic and abiotic constraints. Diseases are the major impediments to production of pulses among the various biotic and abiotic stresses responsible for low productivity (Malik and Bashir, 1992). *Mungbean yellow mosaic virus* (MYMV) is one of the major threats to successful mungbean production. It has been found widely in India, Sri Lanka, Pakistan, Bangladesh, Papu New Guinea, Philippines, and Thailand (Honda et al., 1983; Chenulu and Verma, 1988; Malik and Bashir, 1992; Jones, 2003). The disease appears as small yellow spots on young leaves, large irregular green and yellow mosaic, and high stunting of emerging trifoliate leaves. Occasional puckering of the green area, as well as increase in size of yellow areas in subsequent emerging leaves until they become completely yellow in some plants are also observed. Pods become stunted and curled, and frequently contain small immature seeds (Malik and Bashir, 1992; Nene, 1973). It is most destructive during the summer season (Malik et al., 1986).

An important aspect of integrated disease management of MYMV is the use of resistant varieties. However, for a successful breeding program, a reliable screening technique is a prerequisite. MYMV is reported to be transmitted by a vector whitefly, *B. tabaci*, but not by seed, soil and mechanical inoculation (Naire and Nane, 1973; Ahmad and Harwood, 1973; Honda, 1983). Study of resistance/susceptibility is believed to be rather difficult and laborious because of the involvement of vector and the efficiency of transmission, acquisition period, persistence, and semi-persistence nature of viruses, as well as the host-vector virus interactions. However, these do not pose any problem in graft inoculated (artificially transmitted) viruses. Grafting involves the union of cambial layers of stock and scion, either of which might be infected by a virus (Matthews, 1970; Akhtar et al., 2001). Authors of this study believe that to date, no graft inoculation method for the transmission of MYMV in screening mungbean germplasm has yet been standardized. This study aimed at developing and standardizing a graft inoculation method that can be used to test mungbean germplasm for resistance to MYMV under field, as well as screenhouse conditions.

Materials and Methods

Materials used for this technique were mungbean plants (healthy and MYMV infected), scalpel (with blade), parafilm, test tubes, fine string (to tie the test tube to the grafted stem), distilled water, and beaker.

Four to six seeds of MYMV susceptible cultivar 'Kabuli Mung' were sown in 15 earthen pots, 12 inches in diameter, under insect-free conditions in a screenhouse on 08 May 2003. MYMV-infected dried leaves have been deposited to the Plant Virus GenBank (http://www.virusbank.org). These pots were filled with soil taken from the experimental area of the Nuclear Institute for Agriculture and Biology (NIAB), Pakistan. Thinning was done by having one plant

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per pot 10 days after germination of seeds.

Results and Discussion

Standardized graft inoculation method. Four-week-old plants were selected for graft inoculation. One sliced cut 1-2 cm long and 0.1-0.2 cm deep was made 1-2 inches below the growing tip on the main stem of the test plant. An 18 cm long trifoliate branch having the same thickness as that of the test plant and showing 40-50% yellow mosaic symptoms was detached from naturally infected mungbean plants. A similar cut (as in the test plant) was made on this branch and corresponding cut surfaces were brought together and tied with parafilm to avoid drying and to stop the entry of air (Fig. 1). Care was taken to bring the corresponding cambium surfaces into contact. This stem was then placed in a test tube 2 cm in diameter and 16 cm in length, filled with distilled water. Distilled water was changed daily at an interval of 12-13 hours for 7 days (according to the requirements). After 7 days, the plant was removed from the test tube and observed daily for its success. Another experiment following the same methodology was also done. The only difference was the use of scion with complete chlorosis (yellowing) of trifoliate leaves instead of infected trifoliate leaves with 40-50% leaf mosaic symptoms.

The procedure adopted in this study provides a rapid method for the transmission of MYMV to screen mungbean germplasm for resistance. Disease transmission and success of grafting was 100% in case of plants grafted with MYMV infected trifoliate showing 40-50% infection (Table 1). Graft inoculated plants started to show symptoms within 12-14 days post inoculation as bright yellow spots on the new emerging trifoliates. Symptoms obtained were similar to those of infected mungbean plants used for grafting (Fig. 2). They showed

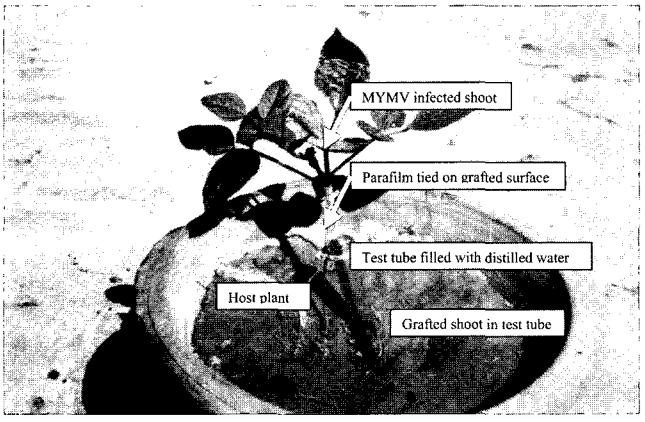

Fig. 1. Bottle shoot grafting method for the transmission of *Mungbean yellow mosaic visus.*

Table 1. Grafting success and disease transmission of the graft inoculation method for the screening of MYMV under screen-house condition

Plant No.	Grafting success[a]	Disease transmission	Latent period[b] (Days)	No. of days to complete chlorosis and necrosis
1	+	+	12	22
2	+	+	13	24
3	+	+	13	24
4	+	+	14	24
5	+	+	13	26
6	+	+	13	26
7	+	+	14	24
8	+	+	12	22
9	+	+	13	25
10	+	+	13	23
Average	100%	100%	13	24

[a] Success of grafting was + when grafted stem (scion) survived for more than 10 days after grafting.
[b] Time taken for disease appearance.

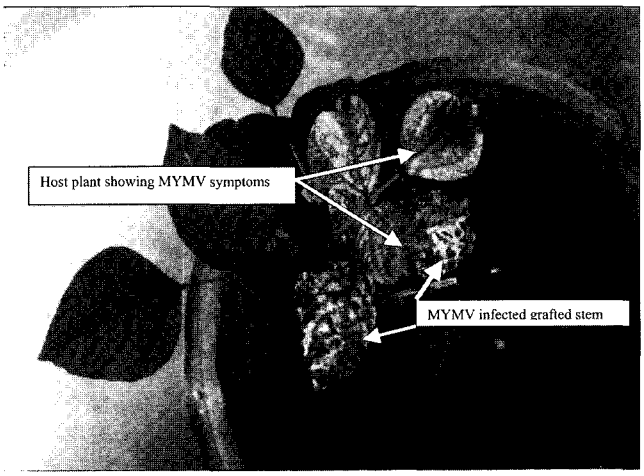

Fig. 2. Upper view of the grafted plant after 16 days.

severe chlorosis and necrosis after 22-26 days of grafting. Observations showed that during the first 3 days of grafting, scion needed more water. When no water was added, the grafted stem utilized all the water in the tube and, as a result, grafting failed or took more time to recover. It is therefore suggested that for excellent results, distilled water must be added at least for the first 3 days.

Plants grafted with completely yellow trifoliate leaves showed no symptoms. This was due to the complete necrosis of the grafted (scions) trifoliate leaves following their death within 3-6 days post grafting. Therefore, it is suggested to use only newly emerging trifoliate scions with 40-50% bright yellow spots as shown in Figure 1.

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