

Cowpea mild mottle virus could not be detected by ELISA in soybean and groundnut seeds in Indonesia

NICO M. HORN¹, NASIR SALEH and YULIANTORO BALIADI

Malang Research Institute for Food Crops, P.O. Box 66, Malang, 65101, East Java, Indonesia

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Abstract

Using ELISA to determine whether cowpea mild mottle virus (CMMV) was present in soybean and groundnut seeds, the virus was not detected in 4144 seeds harvested from seven CMMV-infected soybean genotypes and in 214 seeds collected from CMMV-infected groundnut plants (cv. Gajah). These results, together with those of other researchers, suggest that, under the conditions tested, CMMV is not transmitted by seed. This in contrast to what is generally accepted and published in reviews.

Additional keywords: seed transmission, *Glycine max*, *Arachis hypogaea*.

Iwaki et al. (1986) mentioned the occurrence of CMMV in Indonesia. It was, however, only in 1989 that its presence in groundnut and soybean in Java was unequivocally documented (Nasir et al., 1989). In Indonesia, CMMV is an important virus in soybean crops (Horn et al., 1991). In groundnut, however, it is of much less importance; peanut stripe virus appears to be the major threat (Nasir et al., 1989). In the future, the incidence of the CMMV in soybean is expected to increase because of the increasing population of its whitefly vector (*Bemisia tabaci*).

Brunt and Kenten (1973) reported a seed transmission level of 92% (33 out of 36 seeds) in soybean. Iwaki et al. (1982), however, found 0.9% seed transmission in soybean (one out of 110 seeds) and Thouvenel et al. (1982) found 0.5% (one out of 200 seeds) and 8.3% (five out of sixty seeds) for two soybean cultivars. Iizuka et al. (1984) grew out 500 soybean seeds from CMMV-infected plants, but none of the seedlings showed typical disease symptoms. They also grew out 150 groundnut seeds from CMMV-infected plants, but none showed typical symptoms (Iizuka et al., 1984). At the International Institute of Tropical Agriculture (IITA, Nigeria) 25 soybean genotypes were tested for seed transmission of two CMMV strains. A maximum of 550 seeds from each genotype was planted in trays, but no infection of seedlings was observed (IITA, 1987).

In the study reported here, ELISA was used to determine whether CMMV was present in the seeds of Indonesian soybean and groundnut cultivars. The mother plants were either naturally infected with CMMV or mechanically inoculated with an

¹ Present address: ICRISAT, Patancheru, Andhra Pradesh 502 324, India

isolate of CMMV from soybean (Horn et al., 1991). Leaves and seeds were tested by direct antigen coating-ELISA as described by Hobbs et al. (1987), using penicillinase-conjugated antibody (Sudarshana and Reddy, 1989), because of its convenience for visual scoring of the results. The CMMV antiserum was applied in a 1:500 dilution.

Seeds were tested for CMMV by cutting a small slice from each, grinding five slices from different seeds in 2 ml carbonate buffer and adding 200 μ l of the extract to an ELISA-plate well. In the case of soybean, seed coats were removed, because, as found in preliminary tests, their contents may give a positive reaction. The presence of the virus in the seed coat, however, is not likely to result in seed transmission.

1845 seeds of soybean cv. Wilis were tested, 1172 from naturally infected plants and 673 from mechanically inoculated plants. Furthermore, a total of 2299 soybean seeds from four Indonesian cultivars (Gallunggung, Lokon, Tidar and MLG 2675) and two breeding lines mechanically inoculated with CMMV were tested. For groundnut, 36 seeds from naturally infected plants cv. Gajah and 178 seeds from mechanically inoculated plants of the same cultivar were tested.

None of the 4144 soybean seeds and none of the 214 groundnut seeds reacted positively in ELISA when tested with CMMV antiserum. All mother plants, however, contained the virus from an early stage of development, as was shown by positive reactions in ELISA with CMMV antiserum.

The failure to detect CMMV in seeds by ELISA does not necessarily prove that CMMV is not seed transmitted. The seed transmission rate could be very low or the virus could have been present in the seeds at an amount below the detection level of the ELISA used. Growing-out tests would give a better assessment of seed transmission of CMMV. Such tests could, unfortunately, not be reliably performed due to uncontrollable whitefly infestations in the greenhouse where the plants were grown.

The results of our study do not provide direct evidence against seed transmission of CMMV. However, these results together with those of the extensive study done at IITA (IITA, 1987) and the test conducted by Iizuka et al. (1984) show that the general view that CMMV is seed transmitted should be reconsidered.

Brunt and Kenten (1973), Iwaki et al. (1982) and Thouvenel et al. (1982) found seed transmission of CMMV, using other strains and other cultivars. In these studies only growing-out tests were applied, but no special precautions were reported to prevent accidental contamination with CMMV. The best test would be the one that combines growing out tests, in the absence of whiteflies, with testing of the seedlings by ELISA.

Most reviews mention that CMMV is seed transmitted (CMI/AAB, 1974; Boswell and Gibbs, 1983) based on the results of Brunt and Kenten (1973). They performed their growing-out tests in the UK, thereby minimizing accidental contamination by whiteflies. However, based on all data available from the literature, it is better to state that CMMV is seed transmitted in certain combinations of virus strains and soybean cultivars, than the general statement that CMMV is seed transmitted in soybean. This is especially so, since seed transmission of CMMV apparently ranges from 0 to 92%.

If CMMV is not seed transmitted in Indonesian cultivars, then seeds are not the primary source of infection in soybean fields in Indonesia. Leguminous weeds and other crops are probably more important as primary sources of infection.

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