OCCURRENCE OF AN UNIDENTIFIED POTYVIRUS OF SOYBEAN IN TAIWAN

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ABSTRACT

In surveys conducted by AVRDC, potyviruses were frequently isolated from soybean in Taiwan. Preliminary studies and serological tests indicated that these isolates were not SMV, the only potyvirus so far reported from soybean in this country. The artificial host range, which was almost identical for these isolates, was largely confined to the leguminous family, including economically important crops such as French bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata* ssp. *unguiculata*), yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis*) and azuki bean (*Vigna angularis*).

Myzus persicae transmitted the virus in a non persistent manner. Seed transmission was found to occur in soybean and cowpea. Serological tests indicated a close relationship with azuki bean mosaic virus (AzMV), peanut mosaic virus (PMV-TARI), blackeye cowpea mosaic virus (B1CMV) and certain strains of bean common mosaic virus (BCMV).

Resistance has been found in AVRDC soybean germplasm accessions and breeding lines.

Introduction

Virus diseases constitute a threat to soybean production in the tropics (Goodman and Nene, 1976) and are considered one of the major factors for low average yield of this crop (Sinclair, 1982).

More than 50 viruses are known to infect soybean, but only about 20 of these occur naturally on soybean (Boswell and Gibbs, 1983; Hampton *et al.*, 1978). The most common virus of soybean, soybean mosaic virus (SMV) occurs worldwide, particularly in tropical areas where cases of 100% infection have been reported (Sinclair, 1982). Other viruses reported from soybean in tropical and subtropical areas are tobacco streak virus (TSV), cowpea mosaic virus (CPMV), bean pod mottle virus (BPMV), soybean stunt virus (SSV), soybean dwarf virus (SDV) and mungbean yellow mosaic virus (MYMV) (Boswell and Gibbs, 1983; Sinclair, 1982).

Breeding soybean lines with resistance to virus diseases is one of the objectives of AVRDC's Soybean Improvement Program. In previous years, research had only focused on SMV. Several SMV strains have been isolated from soybean in Taiwan and are being used for resistance screening (AVRDC, 1984).

During surveys of the major soybean production areas in Taiwan conducted from 1981-1985 for the search of naturally occurring SMV strains (AVRDC, 1984, 1985), several filamentous virus isolates were recovered from soybean which were not SMV, the only potyvirus so far reported from soybean in Taiwan (Anonymous, 1973; Murayama and Han, 1971). Three typical virus isolates, PN, PM and 74, are presently under investigation for characterization and to determine their importance in Taiwan and other Southeast Asian countries in order to make an assessment as to whether they should be included into AVRDC's virus resistance breeding program. Some of these studies are reported here.

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Distribution in Taiwan

An islandwide survey was conducted from 1981 to 1985 in Taiwan to determine the frequency and distribution of this virus as well as of soybean mosaic virus (SMV). The major soybean growing areas in Pingtung, Taichung and Hualien, as well as AVRDC fields, were surveyed. A total of 846 leaf samples was taken from plants with typical virus symptoms such as mosaic, mottle, leaf deformation, yellowing and necrosis, but also from symptomless plants. They were examined by ELISA using antiserum to SMV and an antiserum prepared against one of the virus-isolates, PN. Seventy-four (8.7%) of the 846 leaf samples were found to be infected with SMVand fourty-six (5.45) with the unidentified virus. Both viruses were present in each of the surveyed areas (AVRDC, 1983, 1984).

Host range

One to several cultivars of 35 plant species were tested for susceptibility to the three above-mentioned isolates. Five to ten test plants were inoculated and were kept for four weeks in the greenhouse for symptom expression. Both inoculated and non-inoculated leaves were tested by ELISA regardless of symptoms.

The host range was found to be almost identical for the three isolates and was largely confined to members of the legume family, including economically important crops such as *Glycine max, Phaseolus vulgaris, Vigna unguiculata,* and *V. unguiculata* ssp. *sesquipedalis.*

Plant species which were infected systemically were: *Cassia occidentalis, Cyamopsis tetragonoloba, Dolichos lablab, Glycine max* cvs. Bragg, Lee, Stuart, Jupiter, Rampage, Davis, York, Marshall, Ogden, Buffalo, Palmetto, Iwate Wasekurome, Toyosuzu, Bansei, Tainung No. 3, Tainung No. 4, Taita Kaohsiung No. 5, HL-1, Kaohsiung No. 3, Kaohsiung No. 8, Kaoshiung No. 9, Mou Dou 205, Chung Hsing No. 1, Chung Hsing No. 2, Chung Hsing No. 3, Ryokkoh, Ryuhyo, Talien-tou, AVRDC Accessions G2, 2015, 2021, 2038, 10134 and 10446, AVRDC Advanced Glycine Selections AGS 1-8, 10-18, 20-66, 203, 216, *Phaseolus acutifolius* PI 200749; *P. lathyroides; P. lunatus* TPL 240; *P. vulgaris* cvs. Bountiful, Top Crop, Harvester, Kentucky Wonder Waxpole, Kentucky Wax Runner, Black Turtle Soup, Black Turtle I, Black Turtle II, Widusa, Dubbele Witte, Saxa, Redlands Greenleaf C, Redlands Greenleaf B; *Vigna angularis* cvs. Jansen, Odate No. 1, Pingtung native, Kaohsiung No. 1, Kaohsiung No. 2, Kaohsiung No. 3; *V. mungo* AVRDC Acc. 3115; *V. unguiculata* ssp. *unguiculata* cvs. Blue Goose, Early Ramshorn, Blackeye, TVU 1582; *V. unguiculata* ssp. *sesquipedalis* cvs. Bogor 1, Local Variety Yardlong I, Tainung No. 3, Kaohsiung Green Pod, and FTHES CP 57, FTHES CP 72, FTHES CP 22, FTHES CP 1, FTHES CP 10.

Nicotiana benthamiana, N. clevelanddii and *Chenopodium quinoa* were the only non leguminous species which were infected systemically.

On *Glycine max* systemic symptoms often consisted of mosaic, deformation, and necrosis. On *Phaseolus vulgaris* the reactions of all three isolates were generally very severe, with necrosis and malformation often leading to death of the plant.

Although the symptoms produced by the three isolates were similar on all inoculated hosts, it was observed that systemic symptoms of isolate PM were generally milder than those of the other two isolates.

P. vulgaris cvs. Monroe, Black Turtle, Pinto III, Jubila, Sanilac, and *Chenopodium amaranticolor* had local lesions without systemic invasion.

Symptoms were not observed and the virus was not recovered from *Arachis hypogaea* 'Tainan 9', 'Tainan 4', *Capsicum annuum* 'Yolo Wonder', *Cucumis melo* 'Known You', *Cucumis sativus* 'Chicago Pickling', *Datura stramonium* 'R. Fulton Strain'; *Glycine max* cvs. Corsoy, Tokyo, Virginia, Hill, Kwanggyo, HLS, CNS, Mou Dou 205, Tainung No. 15, Gun Tsuru, AVRDC Accessions G-5, 38, 260, 270, 288, 311, 358, 394, 452, 453, 519, 1051, 1096, 1300, 1601, 2444, AVRDC AGS Nrs. 9, 19, 103, 112, 115, 129, 147, 174, 185, 209, 214, 218; *Lycopersicon esculentum,*

'Marglobe', 'Fukuja No. 2', *Nicotiana glutinosa, N. tabacum* 'Samsun', 'Xanthi', *Ocimum basilicum, Petunia hybrida* 'King Henry'; *Phaseolus vulgaris* cvs. Redlands Greenleaf C, Redlands Greenleaf B, Monroe, Amanda, Immuna, Michelite 62; *Physalis floridana, Pisum sativum* 'Perfected Wales', 'Dark Skin Perfection'; *Spinacia oleracea* 'Bloomsdale Long Standing'; *Tetragonia expansa, Trifolium pratense* 'Kenland', *T. repens* 'New Zealand', *Vigna radiata* 'Oklahoma' and AVRDC Accessions G-2010, 2773, and 1111.

Transmission

Seed transmission was tested for the 3 isolates in several local soybean, yardlong and azuki bean cultivars. Two hundred seeds from mechanically inoculated plants were sown within 1 month after harvest in an insect proof screenhouse and the seedlings were visually checked for virus symptoms at 14–20 days after emergence. Both plants with clear symptoms and those with no or very mild symptoms were tested by ELISA for presence of virus.

Table 1 Seed transmission of the 3 soybean virus isolates PM, PN and 74 and germination rate of the virus infected seeds

Host	PM		PN		74	
	Germination	Seed transmission	Germination	Seed transmission	Germination	Seed transmission
	%					
V. unguiculata ssp. *sesquipedalis*						
CP 1	97	11.3	96	17.2	96	17.8
CP 10	97	2	96	10.1	96	11.5
CP 22	95	3.1	NT 1/	NT	NT	NT
CP 40	98	0	98	0	98	0
CP 44	88	0	95	0	95	0
CP 57	98	1	NT	NT	NT	NT
CP 72	98	0.5	96	0	100	0
V. angularis						
Pingtung Native	88	0	85	0	NT	NT
KS 1	89	0	88	0	73	0
KS 2	95	0	72	0	86	0
KS 3	88	0	96	0	NT	NT
G. max						
TN-4	69	1	63	0	65	0.7
TK-5	63	3	66	0	73	0
KS-8	72	0	69	0	78	0
KS-9	84	1	82	2.4	76	0
Bragg	69	0	71	0	70	0
Toyosuzu	78	0	41	0	58	0
AGS 28	92	0	72	0	75	0
AGS 229	98	0	81	0	71	0

1/
NT = not tested.

The results shown in Table 1 demonstrate that the 3 virus isolates were transmitted through seed of yardlong bean and soybean but not through the 3 azuki bean cultivars tested. In the 8 soybean lines, a low rate of seed transmission was found, ranging from 0.7 to 3%. Whereas in yardlong bean seed transmission was higher, ranging from 0.5 to 12.8%.

Aphid transmission tests were conducted for the three isolates with virus-free *Myzus persicae,* reared on healthy *Nicotiana tabacum* 'Xanthi'. Ten groups of five to ten apterous aphids were used. After a 4 h fasting period, the aphids were allowed a 5 min acquisition access feeding on a diseased *Glycine max* cv Tainung No. 4 plant, followed by an inoculation access feeding period of 1 h on a virus-free seedling of the same cultivar.

It was shown that all three isolates were transmitted by *Myzus persicae* in a non persistent manner.

Serology

Antiserum against isolate PN was produced in a rabbit by three intramuscular injections of purified virus. The virus isolates were tested by double antibody sandwich (DAS-ELISA) (Clark and Adams, 1977) against antisera to soybean mosaic virus (SMV-C), bean common mosaic virus (BCMV strains NL5 and NY15), the Florida isolate of blackeye cowpea mosaic virus (BlCMV-Fla), azuki bean mosaic virus (AzMV), the Moroccan isolate of cowpea aphid-borne mosaic virus (CAMV-Mor) and against peanut mosaic virus (PMV-TARI), isolated from peanut in Taiwan (Chang, 1980).

Data shown in Table 2 indicate that the strongest reaction of each of the soybean isolates was obtained with homologous antisera and antisera to AzMV and PMV TARI, followed by BlCMV-Fla antiserum. Weak reactions were produced with SMV-C antiserum. One of the isolates, PM, also reacted with BCMV-NL 5 antiserum. No reaction was obtained with CAMV-Mor and BCMV-NL5. In reciprocal tests, AzMV also reacted strongly with antisera to PN and 74 and weakly with antiserum to BlCMV. BlCMV-Fla reacted less strongly with antisera to PN and 74 than with that to AzMV. Weak reactions were produced by PN and 74 and also by AzMV with SMV ATCC. Interestingly, BCMV-NY15 gave strong reactions with BlCMV, AzMV antisera and PN antisera and a weak reaction with 74 antiserum. There were also no cross-reactions, when the BCMV strains NL-5 and NY-15 were tested with their heterologous antisera in DAS ELISA.

Table 2 Direct ELISA reactions of isolates PN, PM and 74 with homologous antisera and with antisera to other legume potyviruses

	Antisera								
Antigens	SMV-C	BCMV NL5	BCMV NY15	CAMV Mor	BlCMV	AzMV	PMV TARI	PN	74
	1/								
PN	+	–	–	–	++	+++	+++	+++	+++
PM	+	+	–	–	++	+++	+++	+++	+++
74	+	–	–	–	++	+++	+++	+++	+++
SMV-ATCC	+++	+	–	–	–	+	NT	+	+
BCMV-NL5	–	+++	–	–	+	–	NT	–	–
BCMV-NY15	+	–	+++	–	+++	+++	NT	++	+
CaMV-Mor	+	–	–	+++	–	–	NT	–	–
BlCMV-Fla	+	–	+	–	+++	++	NT	+	+
AzMV	+	–	+	–	+	+++	NT	+++	+++
PVM 'TARI'	NT	NT	NT	NT	NT	NT	+++	+++	++

1/ +++ = Indicates very strong reaction, ++ = strong reaction, + = weak reaction, – = no reaction, NT = not tested.

Discussion

The similar reactions obtained by the three isolates PN, PM and 74 on the 35 plant species tested, suggest that they represent either identical or very closely related strains of the same virus. It was observed, however, that symptoms incited by the PN and 74 isolates were generally more severe than those of isolate PM. This was most pronounced on soybean.

It was not possible to identify the three isolates by their reactions on the 35 different plant species which included many proposed by Hampton *et al.* (1978) for the identification of legume viruses.

The narrow host range of our isolates most closely resembles that of BCMV, BlCMV, AzMV, BCMV and SMV (Boswell and Gibbs, 1983; Hampton *et al.*, 1978; Zettler and Evans, 1972; Tsuchizaki *et al.*, 1970). Both BCMV and SMV however are not known to systemically infect tobacco spp. as isolates PN, PM and 74 do. Of the five above listed viruses, only BlCMV is reported to infect *Nicotiana benthamiana* (Lima *et al.*, 1979). However, the three isolates differ from BlCMV in that they infect *Phaseolus vulgaris* 'Black turtle I', which Provvidenti (1983) reported to be resistant to BlCMV. Our isolates produced local lesions on the inoculated leaves followed by severe necrosis on the non inoculated leaves and subsequent death of the plant.

The three soybean isolates, PN, PM and 74, produced a clearly visible systemic reaction on *Glycine max* Iwate Wasekurome, one of the differential hosts, used by Japanese scientists (Iizuka, personal communication) to distinguish AzMV from BlCMV. Only AzMV produces a systemic mosaic on this soybean cultivar whereas the latter produces only a local latent infection.

Furthermore, so far soybean is not known as a natural host of BlCMV. It is, however, reported to be naturally infected by AzMV (Takahashi *et al.*, 1980). Based on their host reactions it would thus appear that the three soybean isolates are more closely related to AzMV than to BlCMV.

The results obtained by direct ELISA generally supported our observations based on host range reactions. No reactions were observed with antiserum to CAMV, a potyvirus whose wide host range is very different from that of our isolates. Strongest reactions were observed with antiserum to AzMV and PMV-TARI, followed by BlCMV and SMV. Such results indicated close serological relationship between our isolates and AzMV and PMV-TARI, as well as BlCMV. Information available from a preliminary characterization of PMV-TARI (Chang, 1982), indicates that PMV-TARI may also represent a virus which is very closely related to AzMV and BlCMV. An interesting finding was that BCMV-NY15 was serologically more related to AzMV and BlCMV than to BCMV-NL5. Because of the strong apparent similarities between BlCMV and AzMV it is suggested to do more cooperative work to clarify whether they are different viruses or strains of the same virus. Clearly the isolates PN, PM and 74 share common characteristics with both AzMV and BlCMV, and BlCMV-NY15, and PMV-TARI, and at this point, with the information available, proper identification of these isolates is not possible.

Our finding that the virus is present in all of the major soybean production areas in Taiwan and the fact that it can infect a wide range of economically important legumes, in some of which it is seed-transmitted, leads us to consider this virus as a potentially serious pathogen. Field experiments are now underway at AVRDC to determine the effect of this virus on yield. Additional surveys will have to be conducted to establish whether and to what extent this virus is also present, in other Southeast Asian countries.

After these studies are completed, a final assessment will then be undertaken to determine whether breeding for resistance to this virus should become an objective of AVRDC's Soybean Improvement Program. Materials resistant to all three isolates have already been identified in preliminary screenings among AVRDC's germplasm collection and AVRDC's advanced soybean breeding lines.

Sources of antisera: SMV-C and BCMV-NY15: H. J. Vetten, Braunschweig, Federal Republic of Germany; BCMV-NL5: D. Z. Maat, Wageningen, Netherlands; BCMV-NY15: G. Mink, Washington, USA; BlCMV: D. Gonsalves, New York, USA and D.E. Purcifull, Florida, USA; CAMV-Mor: D. Gonsalves, New York, USA; AzMV: N. Iizuka, Hokkaido, Japan; PMV-(TARI): C.A. Chang, TARI, Taiwan.

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Discussion

Makkouk, K.M. (ICARDA): It is obvious that the classification of some of the potyviruses is most confusing. I believe that host reactions used for comparative studies of viruses are not reliable for typing a virus. For comparative studies from the host range or serological standpoints, among the large number of isolates of bean common mosaic virus, the NY 15 strain should be used as it appeared to be more related to the virus you described. It could be considered that viruses such as azuki bean mosaic virus, blackeye cowpea mosaic virus and the NY 15 strain of bean common mosaic virus may be different strains of the same virus.

Green, S.K. (AVRDC): Is it justified to call this virus a strain of azuki bean mosaic virus?

Makkouk, K.M. (ICARDA): The virus you described may be a strain of any of the three viruses you compared it with.

Reddy, D.V.R. (ICRISAT): The determination of the polypeptides is not a reliable method of differentiation. I found it interesting to learn that there was a close serological relationship between your isolates and peanut mosaic virus (TARI antiserum). I would like to suggest that you test your isolates with antisera to peanut stripe virus which is closely related to peanut mosaic virus and reacts with blackeye cowpea mosaic virus, azuki bean mosaic virus, bean common mosaic virus and soybean mosaic virus.

Rossel, H.W. (IITA): By comparing isolates with isolates of azuki bean mosaic from Taiwan you may reach a decision on the ecological relationship with the latter.

Answer: Azuki bean mosaic virus is not reported from Taiwan. It is possible that the virus may have been intorduced from Japan through seeds, as it is seed-transmitted.

Dollet, M. (CIRAD): Two methods for the classification of potyviruses are being tested presently in our laboratory, namely the serological relationship between these viruses with antisera made against protein inclusions and the amino acid composition.

Tsuchizaki, T. (Japan): Bean common mosaic virus, blackeye cowpea mosaic virus and azuki bean mosaic virus can be differentiated by their reaction to bean, asparagus bean and azuki bean. Also seed transmission is important for the differentiation as azuki bean mosaic virus is transmitted by seeds of azuki bean unlike bean common mosaic virus and blackeye cowpea mosaic virus. Therefore the virus you described may be closer to blackeye cowpea mosaic virus. In Japan there are 5 potyviruses occurring on soybean: soybean mosaic virus, bean yellow mosaic virus, azuki bean mosaic virus, blackeye cowpea mosaic virus and bean common mosaic virus.

Iizuka, N. (Japan): One may consider that bean common mosaic virus, azuki bean mosaic virus and blackeye cowpea mosaic virus are the same virus with different strains or serotypes.