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Note

Occurrence of *Bean common mosaic virus* (BCMV) Infecting Peanut in Korea

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A virus causing vein banding, sometimes yellow mosaic and rugose symptoms on peanut was prevalent around Suwon, Korea. A survey conducted in the area found disease incidence, depending on cultivar, to range from 79 to 100%. The virus was found to be seed-transmissible in all the five peanut cultivars tested with transmission rates ranging from 2 to 16%. Host range analysis failed to differentiate 9 field isolates collected from different peanuts cultivars showing various symptoms. Inclusion bodies such as scroll, pinwheel and long laminated aggregates induced by the virus in host plant cells were similar to those induced by members of the *Potyvirus* subdivision III. The virus showed < 95% homology with *Bean common mosaic virus* (BCMV), BCMV-BICMV/AzMV strains and only < 91% with *Desmodium mosaic virus*. Based on biological characterization, electron microscopy and molecular analyses of a Korean isolate (Daewon 1), the virus was identified as peanut stripe strain of BCMV.

Keywords **:** *Bean common mosaic virus*, disease incidence, peanut, occurrence, seed-transmission

Bean common mosaic virus (BCMV) is an approved member of the genus *Potyvirus*, family *Potyviridae*. The virus is characterized by flexuous particles of 847-886 nm in length containing a single stranded RNA genome of about 10 kb (Brunt et al., 1996; Morales & Bos, 1998). Peanut-infecting strains of BCMV cause important diseases of legume crops throughout the world. Numerous serological strains and variants with differing symptoms or host range have been described and Peanut-infecting strains were formally called *Peanut stripe virus* (PStV) (Demski et al., 1984; Fukumoto et al., 1986; Higgins et al., 1998; Wongkaew et al., 1990; Xu et al., 1983). The classification of these viruses has been partially simplified by comparisons of nucleotide sequences, usually in the coat protein or 3'-untranslated region (UTR). Furthermore, phylogenetic analysis of legume-infecting potyviruses unequivocally placed PStV within the BCMV group of viruses (Berger et al., 1997; McKern et al., 1992; Saiz et al., 1994; Teycheney et al., 1994; Vetten et al., 1992).

The BCMV subgroup was previously subdivided into two groups named serotypes A and B based on serological differences. Vetten et al. (1992) proposed that these are in fact distinct *Potyvirus* species. Recently, the necrotic serotype A strains have been renamed *Bean common mosaic necrosis virus* (BCMNV) while the mosaic serotype B strains retained the name, BCMV (Khan et al., 1993; Mink et al., 1994). The latter include: *Azuki bean mosaic virus* (AzMV), *Blackeye cowpea mosaic virus* (BlCMV), *Cowpea vein-banding mosaic virus*, *Peanut blotch virus*, *Peanut stripe virus* (PStV) and some isolates from soybean. Despite the high level of biological variability between PStV strains, the level of variability among the CP sequences of PStV from Thailand, Indonesia and the USA was found to be comparable to that observed between other potyviruses (Fukumoto et al., 1986; Higgins et al., 1998).

A peanut (*Arachis hypogaea* L.) disease causing striping and mosaic symptoms was first observed in Georgia, USA in 1982. The peanuts had been raised from seeds imported from China where the disease caused severe yield reductions. The virus was reported to have been introduced into India through the same means (Demski et al., 1984; ICRISAT, 1988). The causal virus is both seed-borne and aphid-transmitted in a non-persistent manner. Depending on the peanut cultivar and stage of development, seed transmission rate varies from less than 1 to 50%. Plants infected early in the growing season may have greater yield losses than those infected late. Moreover, the infected

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plants produce smaller seeds than non-infected ones (ICRISAT, 1988). Following biological, electron microscopy and UTR sequence analyses of a virus causing vein banding disease in peanut we report here the characterization of the virus strain and propose the name BCMV-peanut stripe strain (BCMV-PSt).

A survey of peanut-infecting strains of BCMV was carried out in National Crop Experiment Station (NCES), Suwon, Korea. The survey was conducted by inspecting the crops for the disease symptoms. Disease incidence was calculated as the number of plants showing BCMV symptoms relative to the total number of plants observed. Nine field isolates from peanut cultivars, Saedul, Daepung, Shingang, Daewon 1, Daegang, Namdae 1, ShinDaegang, Daewon 2 and Namdae 2, showing different symptoms were collected and maintained in *Nicotiana benthamiana* plants throughout the study. Seeds of Namdae, a high seed transmission peanut cultivar, were used to observe the effect of spatial distribution in the field. The number of infected plants was analyzed four times by visual inspection, ELISA and electron microscopy (EM). To test seed transmission rate, 70-136 seeds of each of 5 peanut cultivars obtained from NCES were sown in trays of sterilized soil in an insect-free greenhouse maintained at 20-25°C with 12-15 h light period. The seedlings were inspected for disease symptoms for 30 days after emergence and analyzed for the virus presence by ELISA.

In the field, disease incidence depending on cultivar ranged from 78.8 to 100% with cultivar Daegang, Namdae, Daewon and Daepung having the highest incidence while Saedul had the lowest (Table 1). Disease incidence in field trial plants at 30 days after planting was 10.7%, however, the incidence increased to 24.3, 31.4 and 100% 39, 45 and 52 days after planting, respectively (data not shown). All the five peanut cultivars tested were found to transmit the virus by seed with transmission rate ranging from 2 to 16%. Of the 515 plants that emerged, 35 (6.3%) were virus-infected. Peanut cultivar Aul had the highest rate of seed transmission (15.7%) while ShinDaegang had the lowest (Table 2). Seed-transmission was more evident in small and colored seeds than in large and non-colored ones.

Table 1. BCMV infection rates of different peanut cultivars in the field

Cultivar	No. of plants		Infection rates
	Investigated	Infected	
Wang	81	79	97.5
Daegang	84	84	100
Namdae	86	86	100
Daewon	45	45	100
Aul	67	66	98.5
Daepung	87	87	100
ShinDaegang	83	75	90.4
Shingang	88	77	87.5
Saedul	85	67	78.8
	706	666	94.3

Table 2. Seed transmission rates of peanut-infecting virus

Cultivar	No. of Plants		Infection rates (%)
	Investigated	Infected	
ShinDaegang	136	2	1.5
Daewon	77	5	6.5
Wang	116	9	7.8
Aul	70	11	15.7
Namdae	116	8	6.9
Total	515	35	6.8

To determine the infectivity of virus isolates and the symptoms induced on test plants, 5-10 seedlings from each of the species, *Chenopodium amaranticolor*, *C. quinoa*, *N. benthamiana*, *N. tabacum* cv. 'Bright yellow', *N. tabacum* cv. 'Xanthi-nc', *N. tabacum* cv. 'Samsun', *Physalis floridana*, *Petunia* spp., *Datura stramonium*, *Tetragonia expansa*, *Sesamum indicum* L., *Perilla frutescens*, *Impatiens balsamina*, L., *Zinnia elegans* Jacq., *Cucumis sativus* L. *Cucumis melo* L., *Citrullus lanatus*, *Cucurbita moschata* Duch., *Raphanus sativus* L., *Brassica campestris*, *B. rapa* L., *Chrysanthemum coronarium*, *Phaseolus vulgaris* L., *P. angularis*, *P. radiatus* L., *Vicia faba*, *Glycine max* Merr. and *Vigna sinensis* King, at the 3-5 leaf stage were inoculated by sap prepared by homogenizing infected leaf samples in 0.1 M phosphate buffer, pH 7.0. The test plants were put in an insect-free greenhouse maintained at 20-25°C with 12-15 h light period. Disease symptoms were recorded three times a week for 30 days. Both symptomatic and non-symptomatic plants were verified for the virus infection by back inoculation to *C. amaranticolor* and electron microscopy. The most common symptom observed on the youngest leaves was vein banding, which was attributed to the depression of intervenal tissues causing the veins to be more pronounced (Fig. 1). In addition to vein banding, rugose and yellow mosaic were sometimes observed. However, in peanut cultivars, Shindagang and Saedul, a combination of vein banding and yellow mosaic was more conspicuous than vein banding alone (Table 3). Studies on other hosts revealed that all the nine virus isolates induced similar symptoms of necrotic ring spot, vein clearing and mosaic in the infected test plants. The virus was easily transmitted by sap. A representative isolate Daewon 1 caused systemic mosaic symptoms on *N. benthamiana*, *P. vulgaris*, and *A. hypogaea*. Although the main symptom on

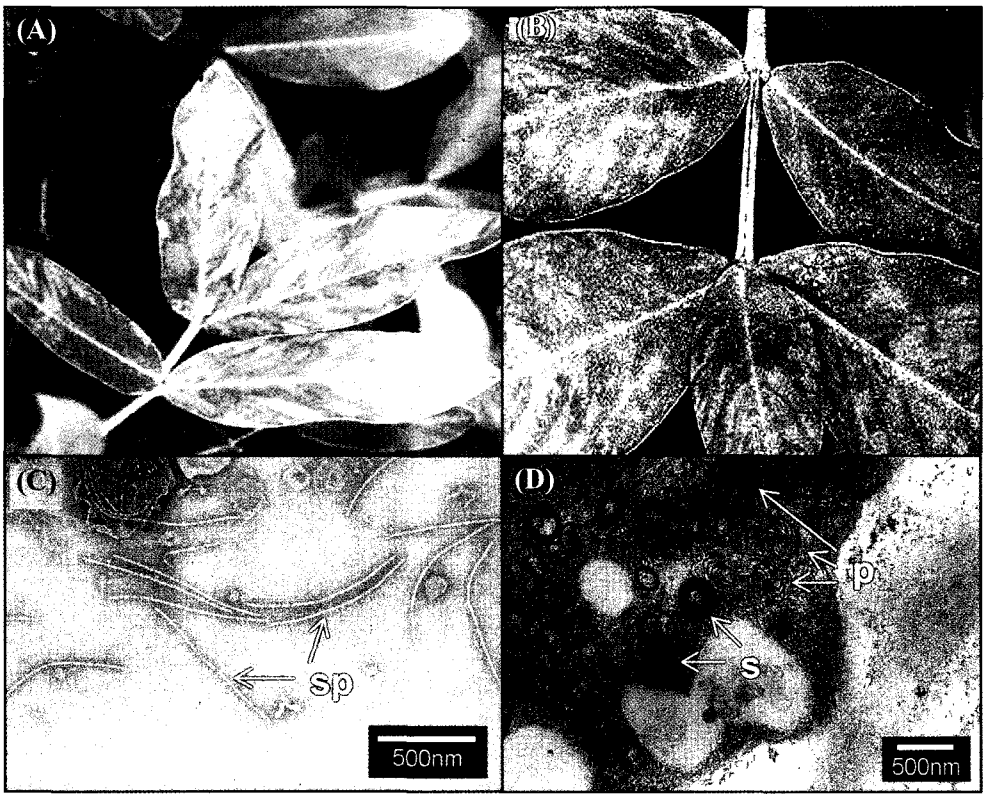

Fig. 1. Virus-infected peanut plants showing (A) yellow mosaic and (B) vein banding symptoms in the field. Electron micrographs showing negatively strained virus particles in crude sap (C) and inclusion bodies (D) Pinwheels (p) and scrolls (s) are marked.

Table 3. Disease symptoms on different peanut cultivars in the field

Cultivar \ Symptom[a]	no	vb	vb,r	vb,ym	ym	Total
Wang	2	57	22	0	0	81
Daegang	0	64	20	0	0	84
Namdae	0	52	34	0	0	86
Daewon	0	42	3	0	0	45
Aul	1	64	0	2	0	67
Daepung	0	78	8	1	0	87
ShinDaegang	8	0	0	49	26	83
Shingang	11	62	0	15	0	88
Saedul	18	25	0	42	0	85

[a]no, no symptom; ym, yellow mosaic; vb, vein banding; r, rugose.

Table 4. Peanut cultivars showing symptoms at 2 weeks post-inoculation with BCMV-peanut isolates

Cultivar	Virus isolate			
	Daegang -vb	Namdae -vb,r	Shindaegang -ym	Daewon -sb
ShinDaegang	–/ym,vb[a]	–/ym,vb	–/ym	–/ym,vb
Wang	–/ym,vb	–/ym,vb	–/ym,vb	–/ym
Aul	–/ym,vb	–/vb	–/ym,vb	–/ym,vb
Namdae	–/ym,vb	–/ym,vb	–/ym,vb	–/ym,vb
Daewon	–/ym,vb	–/ym,vb	–/m,vb	–/ym,vb

[a]m, mosaic; ym, yellow mosaic; vb, vein banding; –, no symptom; Inoculated leaf/Upper leaf.

P. vulgaris was necrotic ring spot, vein clearing was sometimes observed on new leaves. The reactions of peanut cultivars to sap inoculation with the virus were of three types: cultivars Aul and Namdae developed yellow mosaic and vein banding; cultivars Wang and Daewon developed systemic chlorotic mosaic then vein banding, while cultivar Shindaegwang developed yellow mosaic (Table 4).

A disease incidence depending on peanut cultivar was found to be in the range of 79 to 100%. Despite the high incidence, severity was very low as there was no marked reduction in yield. This implies that even when infection pressure is high, farmers could still get high yields and that the peanut cultivars were probably tolerant to the virus. The main symptoms observed in peanut of vein banding and yellow mosaic were not similar to those reported by Higgins et al. (1998) in Thailand. This could be possibly due to differences in peanut varieties used. However symptoms in other host were similar to those found by Wongkaew and Dollet (1990). Field trial results indicate that the disease incidence 30 days after planting was 10.7%, which,

interestingly, increased to 100% after only a short period of 22 days. This indicates that not all seeds at the time of planting had the virus and that mostly virus transmission was or is done in the field probably by aphids. This theory was supported by the presence of a high number of aphids that were roaming the field at the time of the experiment.

The five peanut cultivars used in this study were found to transmit the virus by seed, where transmission rates ranged from 1.5 to 15.7%. These findings agree with those of Xu et al. (1991) who observed that seed transmission rates in peanut could be as high as 50%. In view of these findings and given the fact that an incidence of upto 100% was observed in the field, it can be concluded that some seeds produced from infected plants are actually virus-free. Further experiments are needed to determine the relationship, if any, between time of infection and seed transmission rate and or yield reduction. In most cases seeds that had the virus were small and colored because they had high transmission rate as compared to large non-colored ones. Farmers can exploit this fact by planting larger and non-colored seeds to improve yields.

Indirect-antibody coated enzyme linked immunosorbent assay (indirect-ELISA) and EM observation were conducted essentially as described by Choi et al. (2005) using monoclonal antibodies (MAb) from Agdia (USA). Dip preparations and the sections were viewed under electron microscope LEO 912AB (Carl Zeiss, Germany) at 80 kV. EM examination of crude sap extracts revealed flexuous rod-shaped particles, 730-760 nm long with an average length of 750 nm. The isolates from peanut plants induced typical cytoplasmic inclusion bodies like pinwheels, scrolls and laminated aggregates in cells of *N. benthamiana* (Fig. 2). Infrequently, small bundles of virus-like fibres were found in the nuclei of peanut cells infected with the virus. The virus was easily detected by ELISA and no false positive or negative were found with this method. The presence in crude sap of long flexuous rod shaped filamentous particles of 730-760 nm in length and pinwheels was a confirmation that this virus belongs to the *Potyviridae* family. Furthermore, the BCMV antisera used perfectly detected the presence of the peanut-infecting virus particles, an evidence for a further possible association of the virus with BCMV.

Total RNA was extracted from infected leaf samples essentially as described by Choi et al. (2005). In order to ascertain the taxonomic status of the isolate, 3' terminal region comprising part of the coat protein (CP) gene and 3'-untranslated region (UTR) of the Daewon 1 isolate was amplified essentially as described by Choi et al. (2005). The phylogenetic sequence analysis was conducted as described by Choi et al. (2005) using sequences available from the

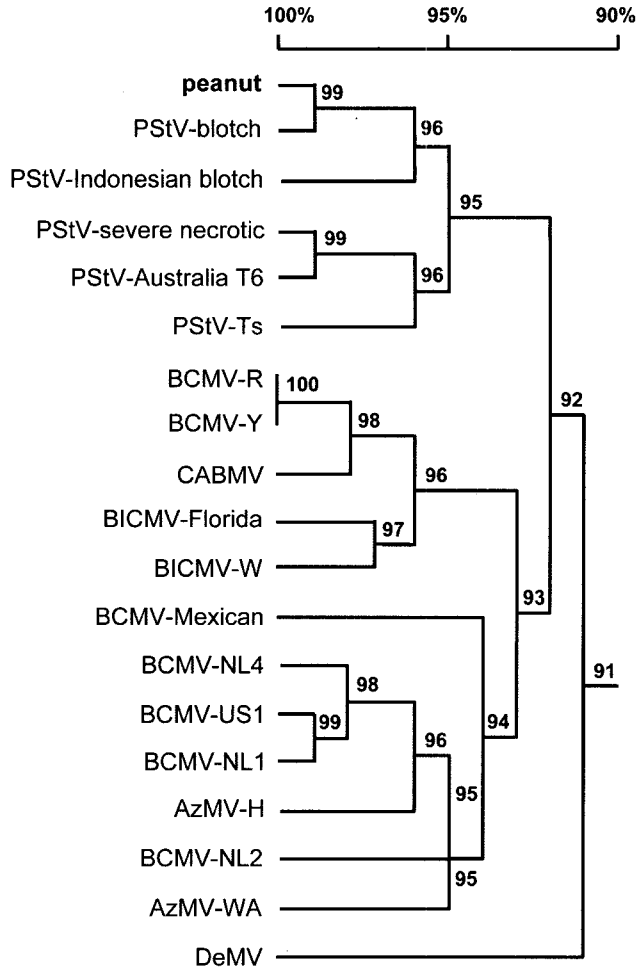

Fig. 2. Phylogenetic tree constructed from nucleotide sequence alignments of the CP/3' UTR fragments of BCMV strains.

database accession numbers U05771 (PStV-blotch isolate), U34972 (Infectious Clone of PStV-blotch isolate), Z21700 (PStV-Indonesian blotch isolate), AF200624 (PStV-severe necrotic strain), Y11773 (PStV-AUSTRALIA T6 isolate), AF063222 (PStV-Ts strain), AJ312438 (BICMV-Y isolate), AJ312437 (BICMV-R isolate), Y17823 (BICMV-Florida isolate), S66253 (BICMV-W isolate), U72204 (Cowpea aphid-borne mosaic virus isolate), L21766 (BCMV-NL4 strain), AY112735 (BCMV-NL1 strain), L12740 (BCMV-US1 strain), L19472 (BCMV-NL2 strain), L11890 (BCMV-Mexican strain), AB012663 (AzMV-H strain), U60100 (AzMV-WA strain), and U23564 (Dendrobium mosaic virus isolate).

The 3' terminal 709 nt sequence comprising 453 nt of the CP and 256 nt 3' UTR with a poly (A) tail were retrieved (Fig. 2). Alignment of CP-3' UTR nucleotide (nt) sequences revealed a varying degree of sequence identity grouping. There was a unique nt motif in the CP of Dendrobium mosaic virus (DeMV) whose affiliation was not known

(data not shown). Moreover, a high degree of sequence homology (> 95%) was evident between the Daewon 1 isolate, the other Asian and USA isolates of PStV. However, when compared to other strains, the Daewon 1 isolate showed < 95% homology with BCMV, BICMV/AzMV and only < 91% with DeMV. Homology analysis of CP-UTR sequences split the strains into 4 groups, PStV, BICMV, BCMV/ AzMV and DeMV groups. The Korean isolate was clustered with PStV-blotch group from the USA. Surprisingly, PStV isolates from the same geographical location did not always have a high percentage of nt identity. For instance, the isolates from Korea shared > 99% nt sequences with USA isolates, < 95% with those from Thailand and < 91% with those from Japan. Phylogenic analysis of the CP/3' UTR nt sequence of isolates from Korea, Indonesia, USA, Mexico, Australia and China showed that they have a common ancestry and that they are strains of BCMV. These findings agree with earlier reports (McKern et al., 1992; Saiz et al., 1994; Vetten et al., 1992), in which it was stated that PStV is a peanut infecting strain of BCMV. Altogether, our results support that the Korean isolate is a peanut-infecting strain of BCMV and the name BCMV-PSt is suggested for the strain.

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