SEED TRANSMISSION OF BEAN COMMON MOSAIC VIRUS

MANDOUR, A. M., H.M.M. ABDELMAKSOUD and MOHGA A. EL-TAHLAWEY

Plant Virology Research Department, Plant Pathology Research Institute, ARC, Giza , Egypt

(Manuscript received 24 January 2013)

Abstract

The virus-distribution and bimodal seed transmission of BCMV in bean cultivars was studied. BCMV was found to be seed transmitted in commercial seed lots of *Phaseolus vulgaris* L. cvs. Royal Nell (25.3%), Savana (13%), and Giza 4 (7.8%). Results showed that mechanically inoculated healthy seedlings (G0) showed typical symptoms of BCMV after 3-4 weeks from inoculation. ELISA test for Generation 1 (G1) detected the BCMV in all plant tissues tested except the cotyledon of cv. Giza 4 and the embryo of cvs. Savana and Giza 4. This finding points to the phenomenon of BCMV bimodal seed transmission.

INTRODUCTION

The bean common mosaic virus was first described by Pierce (1934), In Egypt, it was reported in 1960's (un-published data by Showkria M. EL Attar) and subsequently studied by Abdel-Salam et. al. (1989). Transmission of BCMV through seed plays an important role in the epidemiology of the disease. Therefore the detection of the virus in seed in view of seed quality control assumes a great importance (Hampton, 1967). Seed transmission rates ranged from 3 to 95% (Zaumeyer and Thomas, 1957) depending on the tolerance of bean cultivar and growth stage at which plants become infected (Galvez *et. al.,* 1977).

BCMV may be able to traverse the cell wall between the testa and the suspensor by an unidentified mechanism that does not require plasmodesmata, or that virus may be able to induce formation of new plasmodesmata, thus allowing direct invasion of the embryo (Ekpo and Saettler, 1974). Clearly, both direct and indirect routes of embryo infection occurred in this system. Extensive spread of BCMV through the testa and endosperm was seen only in bean cultivars transmitting BCMV through the seed. In non-seed transmitting cultivars, BCMV caused very limited invasion of the testa and endosperm (Kaiser *et. al.,* 1968, Morales and Castano, 1987).

This study aimed at clarifying the virus-distribution and confirming the bimodal seed transmissibility for (BCMV) under Egyptian conditions.

MATERIALS AND METHODS

Source of virus isolate

Samples from naturally infected bean plants (*P. vulgaris*) grown at Ismailia Governorate and showing typical BCMV symptoms of yellowing, mild mosaic, and malformation were collected and checked against BCMV by indirect-ELISA (Hobbs *et al.,* 1987).

Isolation

Samples reacting positively in ELISA test were used for mechanical inoculation. Single local lesions (Kuhn, 1964) were obtained by mechanically inoculating *Chenopodium quinoa* Willd. One local lesion was separated, ground in phosphate buffer (pH 7.2) and used to inoculate healthy bean plants, which served as a source of BCMV.

Seed transmission

Sets of commercial seed lots of *P. vulgaris cvs.* Royal Nell, Savana, and Giza 4 were tested for the level of virus seed transmission by sowing 100 seed of each cultivar under standard greenhouse conditions. Visual examination of the seedlings for BCMV symptoms was used at the first true leaf stage and examined daily for 4 weeks after emergence. In addition, all the seedlings were tested by indirect-ELISA.

Detection of BCMV in different plant parts

Sets of fifty healthy seedlings of each of the cultivars Royal Nell, Savana and Giza 4 at the first true-leaf stage were mechanically inoculated with BCMV and kept under optimal growth chamber conditions (G0). Two weeks after inoculation, plants were tested by indirect-ELISA and thirty seed were collected at random from the infected mature plants and sown to produce the first vegetative generation 1 (G1). The first true leaves were assayed immediately after expansion. As the plants grew, the leaves, stipule and peduncle were sampled and tested. At the flowering stage, the flowers, stipule and peduncle were excised and tested for the presence of BCMV. Immature and mature seeds were collected randomly and were also tested for BCMV by indirect-ELISA.

RESULTS

Seed transmission

Using 100 seeds of each of three commercial bean cultivars, BCMV was detected according to visual symptoms in 5 seedlings and serologically in 7 out of 90 germinated seeds of *cv.* Giza 4. Ninety-two seedlings of *cv.* Savana was tested for BCMV, 9 seedlings showed BCMV symptoms, while ELISA test confirmed the presence of BCMV in 12 seedlings. In 87 seedlings of *cv.* Royal Nell, 16 gave positive symptoms while 22 ELISA-reacted. Hence and according to the ELISA test, the percentages of seed transmission through different bean varieties were 25.3% for *cv.* Royal Nell, 13% for *cv.* Savana, and 7.8% to *cv.* Giza 4 (Table 1). This indicated that ELISA is more reliable in detecting the virus than visual symptoms under the experiment conditions.

Table 1. Seed transmission of BCMV in seed lots of three commercial Bean cultivars Royal Nell, Savana and Giza 4.

observations	Royal Nell	Savana	Giza 4
No. of seed germinated	87	92	90
No. of infected seedlings upon ELISA test	22 (25.3%)	12 (13.0%)	7 (7.8%)
No. of infected seedlings based on visual symptoms	16 (18.4%)	9 (9.8%)	5 (5.6%)

Detection of BCMV in different plant parts

The mechanically inoculated seedlings Generation 0 (G0) showed typical symptoms of BCMV on leaves 3-4 weeks after inoculation. In plants of generation 1 (G1) indirect-ELISA detected BCMV in all tissues tested except the cotyledon of *cv.* Giza 4 and the embryo of *cvs.* Savana and Giza 4 as shown in Table (2).

Table 2. Detection of BCMV in Bean tissues and organs by ELISA through generation-one of 3 different cultivars.

Seed Generation	Tissue tested	Varieties		
		Royal Nell	Savana	Giza 4
G1	Leaves	* 24 (80%)	21 (70%)	21 (70%)
	Flowers	30 (100%)	27 (90%)	27 (90%)
	Stipule	27 (90%)	24 (80%)	27 (90%)
	Peduncle	27 (90%)	27 (90%)	27 (90%)
	**GS	30 (100%)	27 (90%)	27 (90%)
	Cotyledon	9 (30%)	3 (10%)	0 (0%)
	Embryo	6 (20%)	0 (0%)	0 (0%)

* Number of positive samples out of thirty as tested by ELISA

** Green Seed

DISCUSSION

Results of BCMV occurrence in different plant parts showed that some seedlings of *cvs.* Royal Nell, Savana, and Giza 4 were infected by BCMV with or without recognizable symptoms. This stresses the caution in interpreting the results of seed transmission tests if the results are based on visual symptoms alone. Symptoms in BCMV -bearing seedlings developing from infected seed range from very mild to severe, depending on the virus strain, host plant and growing conditions. Also, symptoms may either develop as the plant grows or fade as the plant approaches flowering (Hampton and Mink, 1975) due to a cogent evidence that some viruses of *potyviridae* are both seed transmitted and seed borne (Kelly, 1997).

Agarwal (1979) reported that BCMV could be detected by ELISA in an average of 49% of bean seed in commercial seed lots, and only 9% of the seed produced by infected seedlings. This can interpret the lower percentage of seeds of infected plants, which express the presence of the virus.

In the present study, BCMV was found to be seed transmitted in cvs. Royal Nell, Savana, and Giza 4, but the level of transmission ranged from high 19% in cv. Royal Nell to lower than 6% in *cv.* Giza4. These differences could be attributed to the seed maturity or virus strain (Provvidenti and Braverman 1976).

The mode of BCMV transmission through seeds of different bean varieties was studied. All fifty inoculated plants (G0) showed the typical BCMV-symptoms. In the (G1) the infected seedlings raised from the seeds randomly collected from each variety under study had clear symptoms of BCMV and was detectable by indirect-ELISA in tissue parts (organs) assayed, except the cotyledon of *cv.* Giza 4 and the embryo of *cvs.* Savana and Giza 4. This result indicate that the infected embryo produced an infected seedling and the transmission of BCMV into the embryo through either pollen or ovule did not occur and therefore seed transmission might be attributed to direct invasion of immature bean seed (Gugielmetti, 1974).

A direct embryonic invasion appears to be more of an enigma in describing such phenomena of BCMV seed transmission which is not confirmed so far. The virus must cross boundary between two generations of the host, simplistic connections to the megaspore mother cell are broken prior to fertilization. Passages of viruses across cell walls in the absence of plasmodesmata in contrary to the dogma of virus cell-to cell movement. Therefore clearly, some unknown mechanism must operate in these cases (Morales and Bos, 1988).

Wang and Maule, (1992) and Wang *et al.,* (1993) described a similar seed born transmission phenomena for Pea Seed borne Mosaic virus (PSbM) for which PSbMV accumulated equally and extensively in the pod tissues of *cv.* Progreta prior to fertilization but was sparse in its distribution in the integument tissues of the ovule. Fertilization stimulated the ingress of the virus into the testa through the peripheral vascular bundle of the immature pea Seed, presumably coordinated with the activation of these tissues as sinks for photoassimilates. PSbMV then spread out of the vascular tissue and through non-vascularized areas of testa. Hence, in host-virus interaction, the virus invasion of vascular areas of testa tissues allow the virus to become seed transmitted. On the other hand, the virus invasion of the non-vascular testa tissues could temporarily block the virus to reach the micropyle region of the testa for seed transmission to occur.

In this Study, the absence of virus in the embryo of *cvs.* Savana and Giza 4 when tested by indirect-ELISA could be attributed to the abundance of virus particles in the integumentary tapetum (the cell layer immediately adjacent to the embryo sac), and in residual integumentary cells of ovary wall (Hunter and Bowyer, 1993) without passing into the cell ovary.

On the other hand, BCMV have been found in plumules and endosperms, but not in the embryo tissues of ungerminated soybean and bean seed and this could be attributed to the development of a callous layer around the maturing megaspore mother cell which might prevent infection of the embryo sac as no protoplasmic

connections were evident between the embryo sac and the tapetum as described by Hunter and Bowyer (1993).

As further complication, BCMV occurred in relatively high concentration in seeds collected from symptomless mother plants before symptoms expression in which virus was not detectable in leaves by serological methods, using ELISA test. BCMV was detected easily within flower parts and seeds of the first generation plants grown from infected seed and this so called "subliminal occurrence" or "eclipse" of virus, in vegetative stages of plant growth, which may also explain the bimodal type of seed transmission of BCMV as described by (Hunter and Bowyer, 1993).

REFERENCES

1. Abdel-Salam, A. M., A. M. El-Sharkawy, M. A. Kararah, M. A. S. El-Kady and Maisa A.E. Awad. 1989. Purification and serological studies on a local isolate of bean common mosaic virus in Egypt. Proc.7th Conf. Microbiol., Cairo, Egypt. pp 370-378. Nov. 19-20.
2. Agarwal, V.K., Y.L., Nene, S.P.S. Beniwal and H.S. Verma. 1979. Transmission of bean common mosaic virus through urdbean (Phaseolus mungo) seeds. Seed Sci. & Technol. 7:103-108.
3. Ekpo, E. J. A. and A. W. Saetter. 1974. Distribution pattern of bean common mosaic virus in Phaseolus Vulgaris . Plant Dis. Rep. 59(12): 939-943.
4. Galvez, G. E., Otoya, M., and LIontop, E. 1977. Determina cionde cepas del virus del mosaic coman del frigol (BCMV). Pro. Amer. Phytopathology. Soc. 4:177 (Abstr.)
5. Gugielmetti, M. H. 1974. A bean variety with resistance to yellow mosaic. (JAP). Insect Prog. Agr. 6:26-27.
6. Hampton, R. O. 1967. Natural spread of viruses infectious to beans. Phytopathology 57(5): 467-481.
7. Hampton, R. O. and Mink, G. I. 1975. Pea seed borne mosaic virus. Number 146. CMI/ABB Description of plant viruses, Kew, Surrey, England.
8. Hobbs, H. A., Reddy, D. V. R., Rasjeshwari, R. and Reddy, A. S. 1987. Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses.Plant Dis. 71, 747-749.
9. Hunter, D. G. and Bowyer, J. W. 1993. Cytopathology of lettuce seeds and seedlings. Journal of Phytopath. 137: 61-72.
10. Kaiser, J.W., Danesh, D., Okhovat, M. & Mossahebi, H. 1968. Diseases of pulse crops (Edible legumes) in Iran. Plant Dis. Reptr 52:687-691.

11. Kelly, J. D. 1997. A review of varietal response to bean common mosaic potyvirus in Phaseolus vulgaris. Plant Var. Seeds 10:1-6.
12. Kuhn, C. W. 1964. Separation cowpea virus mixtures. Phytopathology 54, 739-740.
13. Morales, F.J. & Bos, L. 1988. Bean common mosaic virus. AAB Descriptions of Plant Viruses No. 337. Association of Applied Biologists, Wellesbourne.
14. Morales, F.J. & Castano, M. 1987. Seed transmission characteristics of selected bean common mosaic virus strains in differential bean cultivars. Plant Dis. 71:5153
15. Pierce, W. H. 1934. Viruses of the bean. Phytopathology 24: 87- 115
16. Provvidenti, R. & Braverman, S.W. 1976. Seed transmission of bean common mosaic virus in phasemy bean. Phytopathology 66:1274-1275.
17. Wang, D. and Maule, A. J. 1992. Early embryo invasion as determined in pea of the seed transmission of pea seed-borne mosaic virus. J. G. Virol. 73: 1615-1620.
18. Wang, D., R. D. Woods, A. J. Cockbain, A. J. Maule and A. J. Biddle. 1993. The susceptibility of pea cultivars to pea seed-borne mosaic virus infection and virus seed transmission in the UK. Plant Pathology 42: 42-47.
19. Zaumeyer, W. J. and H. R. Thomas. 1957. A Monographic Study of Bean Diseases and Methods for Their Control. USDA Tech. Bull. 868. 255 pp.

.

الانتقال بالبذرة لفيروس التبرقش العادي في الفاصوليا

ايمن محمد مندور ، هشام محمدين عبد المقصود ، مهجة عبد الرحمن الطحلاوى

قسم بحوث الفيروس – معهد بحوث امراض النباتات – مركز البحوث الزراعية – الجيزة

تم دراسة توزيع وانتقال فيروس التبرقش العادي في الفاصوليا ووجد ان الفيروس ينتقل بالبذور فى الاصناف التجارية لأصناف الفاصوليا رويال نيل (بنسبة 25.3%) ، سافانا (بنسبة 13%) ، جيزة 4 (بنسبة 7.8%). و اظهرت النتائج ان كافة النباتات (الجيل صفر) وعددها 50 نبات لكل صنف و التي تم عدواها بالفيروس النقي قد اظهرت اعراض فيروس التبرقش العادي في الفاصوليا بعد 3–4 اسابيع من العدوى. و اظهر اختبار الاليزا لنباتات الجيل الاول ان فيروس التبرقش العادي في الفاصوليا موجود فى كافة الانسجة المختبرة ما عدا فلقات البذرة لصنف جيزة 4 و اجنة البذور لصنف سافانا و جيزة 4. و هذه النتائج يمكن ان توضح ظاهرة الانتقال الثنائي لفيروس التبرقش العادي في الفاصوليا من خلال البذور.