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Host Range Studies of Bean common Mosaic Virus (BCMV) Infecting French Bean

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ABSTRACT: To ascertain the biological link between BCMV and other host plants, a french bean infecting isolate was physically inoculated into various host plants. 13 host plants out of the 25 host plants examined were shown to be easily infected by the virus. Across plant species, there were differences in the incubation period required for symptom expression typical symptoms of mosaic, mottling and leaf rolling were produced on pea, pole bean and cowpea. The pole bean expressed symptoms after long incubation period (15-18 days). BCMV produced mosaic symptoms on green gram, moth bean, black gram and horse gram. A typical chlorotic and necrotic local lesion symptoms were produced with shorter incubation period on *Glysine max* L. (6-7 days), *Nicotiana rustica* (5-6 days) and *Chenopodium amaranticolor* (4-5 days). To verify the existence of BCMV, every plant was exposed to the Double Antibody Sandwich-Enzyme Linking Immunosorbent Assay (DAS-ELISA). The results demonstrated that the host plants that had distinct symptoms following mechanical sap inoculation had a good response to the antibody-specific.

Keywords: DAS-ELISA, mechanical inoculation, incubation time, and bean common mosaic virus.

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is an important and popular leguminous vegetable crop grown in India and other parts of the world. It is a highly relished vegetable and seed crop in different countries (Salgar *et al*., 2021).

French bean crop is succumb for many viral diseases *viz.*, bean common mosaic virus (BCMV), bean yellow mosaic virus (BYMV), broad bean mosaic virus (BBMV), bean leaf roll virus (BLRV), bean distortion dwarf virus (BDDV), mung bean mosaic virus (MBMV), dendrobium mosaic pot virus (DeMV), bean southern mosaic virus (BSMV), bean pod mottle virus, and bean mild mosaic virus (BMMV) are among the many viral diseases that affect the french bean crop. The bean common mosaic virus is a significant viral disease that causes significant yield losses among all viruses. McDonald initially identified the virus in 1936, and Kulkarni (1973) verified its authenticity. Since the BCMV is thought to spread by aphids, pollen, seeds, sap, and other sources, it has become a significant issue for bean agriculture everywhere the crop is produced (Kennedy *et al*., 1962; Trindade *et al.*, 1984; Puttaraju *et al*., 2004; Kapil *et al*., 2011).

BCMV is one of the virus that affects french beans, has a wide host range and can infect several leguminous crops. Bean common mosaic virus (BCMV) was initially identified in 1917 in *Phaseolus vulgaries* L. in the United States. By 80 per cent lowering the yield and quality of the collected produce, it caused significant economic damage (Drijfhout, 1991). Yaraguntaiah and Nariani reported the BCMV in India in 1963. This virus is the type member of the Potyvirus genus, which is a member of the *Potyviridae* family. According to Drijfhout (1991), the disease can result in considerable yield losses between 50 and 100 percent in a variety of host plants. The BCMV infected plants in a natural setting diseases are typified by systemic signs such as vein banding, mottling, and mosaic signs such as vein clearing, puckering, leaf bending, and uneven leaf lamina (Mangeni *et al*., 2014).

The emerging capacity of viruses is strongly connected with their ability to infect a variety of host plants, or to have a high host range breadth (HRB). Compared to viruses without seed transmission and those with double-stranded genomes (which are nearly mainly RNA), viruses with single-stranded genomes were found to have broader host ranges (Moury *et al*., 2017). It is abundantly evident that the primary drivers of disease transmission are a combination of direct and indirect interactions, with a primary emphasis on research on the host range of viruses (McLeish *et al.*, 2019).

One of a virus's most important characteristics is its host range, which indicates how well suited the virus is to naturally infect other hosts. The vulnerable host must sustain the virus life cycle in order to be an effective member of the host a club of viruses (Fermin, 2018). The best way to transfer BCMV to other host plants, according to a number of researchers, is through mechanical sap inoculation (Morris *et al*., 2006; Bhadramurthy and Bhat, 2009 and El-Kady *et al*., 2014).

Therefore, knowing about biological relationships, such as those found in host range studies of certain viruses,

is crucial to determining their genesis and aids in the development of effective management strategies (Morris *et al*., 2006). In light of this background, the current study has been carried out to examine the host range of BCMV, which infects french beans and other host plants. To do this, DAS-ELISA with an antibody specific to BCMV has been used, along with mechanical sap injection.

MATERIAL AND METHODS

Maintenance of BCMV inoculum. The leaves of young french bean plants showing prominent symptoms of BCMV signs were gathered from the College of Horticulture, GKVK, Bengaluru. The collected samples were mechanically inoculated into french bean (Cultivar Moraleda) plants, and the culture was kept as a stock for future inoculations. The ability of the BCMV to infect several host plants from different families *viz.*, namely *Chenapodiaceae*, *Solanaceae*, *Leguminaceae*, and *Cucurbitaceae*, was tested. Seeds from 25 different host plants were gathered and sown in portrays. seedlings were moved to pots containing soil and sterilized coconut coir pith after 10- 15 days.

Mechanical inoculation. The BCMV culture was kept in the Department of Plant Pathology, GKVK, Bengaluru, and the stock culture was used for mechanical inoculation. Two to three leaf stage healthy seedlings of several host plants were mechanically inoculated with the collected sap after the culture was crushed in a pre-chilled pestle and mortar using phosphate buffer (0.1 M, pH 7.0) with the standard extract, celite (600 mesh) at a rate of 0.025 g/ml of and 0.02% Mercaptoethanol was added.

The inoculum was applied gently on the upper surface of the leaves with a small piece of absorbent cotton wool. The inoculated leaves were washed 1-2 minutes after inoculation to remove the excess of inoculum with a fine jet of distilled water from a squeeze bottle and plants were kept under observation for 15-20 days in the glass house. The potential of the BCMV to infect several host plants from distinct families, *viz.*, namely *Chenapodiaceae*, *Solanaceae*, *Leguminaceae*, and *Cucurbitaceae*, was assessed. Seeds from 25 different host plants were gathered and sowed in portrays, seedlings were moved to pots containing soil and sterilized coconut coir pith after 15 days. Ten plants from each plant species were infected, and one pair of uninoculated plants served as a control. The inoculated plants were kept in an insect-proof glass house and were checked for symptom expression on a regular basis.

Back inoculation test: The purpose of the back inoculation test was to confirm the virus origin. To confirm the viral etiology, the contaminated plant leaves were removed and mechanically re-injected into the propagation host. The back inoculation tests infected leaves were used for the DAS-ELISA serological assay.

Serological assay: Three weeks after inoculation, french bean leaves infected with a virus were examined using DAS-ELISA.

Anti-BCMV antibodies were placed on polysterene plates and incubated for two hours at 37 °C after being diluted in 1:200 coating buffer. Sap was extracted by pestle and mortar the leaves in the extraction buffer, then centrifuged at 8000 rpm for 5 minutes. After the samples sap was removed, it was added to the coated polysterene plate and left to incubate overnight at 4 °C. After adding the anti-BCMV antibody conjugated with alkaline phosphatase (ALP) in 1:200 dilutions, the mixture was incubated for two hours at 37 °C. Next, p-nitrophenyl phosphate (AGDIA, India) was added and allowed to sit at room temperature for one hour. At the conclusion of the test, the color shift confirms the presence of BCMV, and the absorbance values were recorded at 405 nm on an ELISA plate reader (Basavaraj, 2014).

RESULT AND DISCUSSION

13 host plants out of 25 host plants examined had various systemic symptoms, including a mosaic, vein banding, vein clearing, puckering and downward curling of leaves on french bean and field bean at 7-10 days post inoculation. A typical symptoms of mosaic, mottling and leaf rolling were produced on pea, pole bean and cowpea. BCMV produced mosaic symptoms on green gram, moth bean, black gram and horse gram. There was a variation in incubation period taken for expression of symptom by different host plants. The pole bean expressed symptoms after long incubation period (15-18 days). A typical chlorotic and necrotic local lesion symptoms were produced with shorter incubation period on soya bean (6-7 days), tobacco (5-6 days) and *Chenopodium amaranticolor* (4-5 days). The *Solanaceae* family of plants, including brinjal required ten to twelve days of incubation before exhibiting symptoms such as mild mosaic, mosaic, and mottling, in that order. The *Leguminaceae* family of host plants required an incubation period ranging from eight to twelve days in order to expression of various symptoms, such as mosaic, mottling, and mung bean and pea. Cowpea was found to have mosaic, mottling, and rolling leaves. None of the symptoms were exhibited by the *Cucurbitaceae* family plant species (Table 1 and Fig. 1).

The varying duration of incubation required by host plants to manifest symptoms could be a result of the hosts insufficient reaction to the virus growth and spread, as well as its migration. (Zhang *et al*., 2012). According to Zitter and Murphy (2009) and Chellappan *et al*. (2005), host genotype, plant age, temperature, virus strain, and host type all affect even systemic virus penetration in some hosts. The interplay between host resistance and virus pathogenicity genes determines the symptoms of the virus (Brunt *et al*., 1996; Chung *et al*., 2015). Chickpeas, Lablab beans, cluster beans, lima beans, chilli, tomato, cucumber, or pumpkin were not infected by BCMV.

Table 1: Host range of different hosts of BCMV and its confirmation through DAS-ELISA.

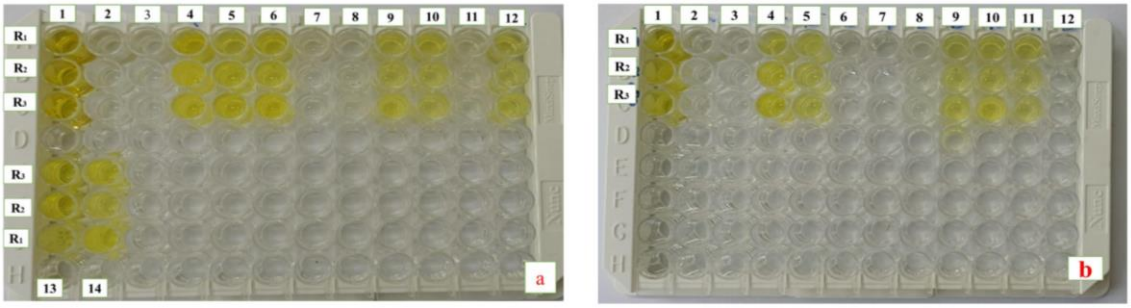
Sr. No.	Plant species inoculated	Family	No. of plants infected	Days post inoculation (dpi)	per cent Infection	Symptomsexhibited	ELISA confirmation	OD value
1	French bean (*Phaseolus vulgaris* L.)	*Leguminaceae*	10	7-10	100.0	M, VB, DC, VC, P	+	2.06
2	Peas (*Pisum sativum* L.)		9	9-10	90.0	M, Mo, LR	+	1.29
3	Green gram (*Vigna radiate* L.)		8	8-12	80.0	M	+	1.36
4	Moth bean (*Vigna aconitifolia*)		2	7-10	20.0	M	+	1.90
5	Pole bean (*Phaseolus coccineus*)		5	15-18	50.0	M, Mo, LR	+	1.95
6	Winged bean (*Psophocarpus tetragonolobus* L.)		0	-	-	-	-	0.59
7	Field bean (*Dolichos lablab*)		9	7-10	90.0	M, VB, DC, VC, P	+	1.67
8	Black gram (*Vigna mungo*)		8	8-12	80.0	M	+	1.55
9	Red gram (*Cajanus cajan* (L.) Millsp.)		0	-	-	-	-	0.46
10	Soybean (*Glysine max* (L.) Merr.)		9	6-7	90.0	CL, NL	+	1.94
11	Horse gram (*Macrotyloma uniflorum*)		2	10-12	20.0	M	+	1.21
12	Cowpea (*Vigna unguiculata* L.)		9	7-10	90.0	M, Mo, LR	+	2.23
13	Groundnut (*Arachis hypogaea* L.)		0	-	-	-	-	0.51
14	Chickpea (*Cicer arientinum*)		0	-	-	-	-	0.47
15	Lima bean (*Phaseolus lunatus*)		0	-	-	-	-	0.39
16	Tomato (*Lycopersicon esculentum* Mill.)	*Solanaceae*	0	-	-	-	-	0.42
17	Potato (*Solanum tuberosum*)		0	-	-	-	-	0.40
18	Chilli (*Capsicum annum* L.)		0	-	-		-	0.51
19	Brinjal (*Solanum melongena* L.		2	10-12	20.0	M, Mo, MM	+	1.11
20	Tobacco (*Nicotiana rustica* L.)		7	5-6	70.0	CL, NL	+	1.62
21	Cucumber (*Cucumis sativus* L.)	*Cucurbitaceae*	0	-	-	-	-	0.44
22	Pumpkin (*Cucurbita moschata*)		0	-	-	-	-	0.35
23	Ridged gourd (*Luffa acutangula* L.)		0	-	-	-	-	0.37
24	Bottle gourd (*Lagenaria siceraria*)		0	-	-	-	-	0.40
25	*Chenopodium amaranticolor*	*Chenapodiaceae*	5	4-5	50.0	CL, NL	+	2.00

No of inoculated: 10 **M-** Mosaic, **Mo**- Mottling, **NL**- Necrotic local lesion, **Cl**- Chlorotic lesion, **P**- Puckering, **VC**- Vein clearing, **VB**- Vein banding, **LR**- leaf rolling, **DC**: downward curling of leaves, **MM:** Mild mosaic, **V+** positive reaction with BCMV specific antisera, **dpi**- days post inoculation.

Similar results were found with Salgar *et al*. (2021) who reported the susceptibility of red gram, french bean, green gram, black gram and pea to BCMV. The number of days taken for symptom expression was varied from 4-18 within plant species. The results are in conformity with the findings of Bhadramurthy and Bhat (2009) who studied the combination of mechanical sap inoculation and sensitive methods like PCR and ELISA have been made accurate determination of host range, strain identification and cultivar differentiation to different virus groups. BCMV was failed to infect selected species of *Leguminosae* and *Cucurbitaceae* plant species *viz.*, *Psophocarpus tetragonolobus*, *Cajanus cajana*, *Cicer arientinum*, and *Arachis hypogacdes*. The host specificity of BCMV isolates infecting *Vigna tahitensis* has already discussed by Grisoni *et al*. (2004) who failed to transmit BCMV isolates to *V. marina* and *Macroptilum lathyrodoides*. DAS-ELISA was used on all of these plants to confirm the presence of BCMV. The results demonstrated that the host plants with various symptoms reacted positively to the BCMV antisera, producing a bright yellow color. The BCMV caused systemic symptoms in several leguminous plant species, including the french bean, pea, green gram, horse gram, field bean, black gram, soybean, cowpea, pole bean, moth bean, and brinjal (*Solanaceae*), although *Chenapodium amaranticolor* and *Nicotiana rustica* only showed necrotic local lesions. The *Cucurbitaceae* plants were not infected with BCMV. DAS-ELISA utilizing BCMV specific antisera verified the presence of BCMV in diverse host plants (Fig. 2a and b).

Plants showing different kinds of symptoms upon mechanical inoculation with BCMV under glasshouse condition (a) French bean (b) Pea (c) Green gram (d) Soy bean (e) Cowpea (f) Horse gram (g) Field bean (h) Black gram (i) Pole bean (j) Moth bean (k) *Chenopodium amaranticolor* (l) *Nicotiana rustica* (m) Brinjal

Fig. 1. Different plant species exhibiting varied symptoms upon mechanical inoculation with BCMV isolate under greenhouse conditions.



(a). 1: Positive control of BCMV infected french bean leaf 2: Buffer control 3: Healthy french bean leaf as negative control 4: French bean 5: Cowpea 6: Field bean 7: Chickpea 8: Red gram 9: Pole bean 10: Moth bean11: Winged bean12: Pea 13: Soybean 14: Green gram
(b). 1: Positive control of BCMV infected french bean leaf 2: Buffer control 3: Healthy french bean leaf as negative control 4: Black gram 5: Chenopodium 6: Tomato 7: Chilli 8: Cucumber 9: Tobacco 10: Brinjal 11: Horse gram 12: Ground nut

Fig. 2. Confirmation of BCMV in different hosts through DAS ELISA.

Similarly, the findings are consistent with the findings of Manjunath *et al*. (2017), who investigated the host range of BCMV by mechanically inoculating several leguminous plants. The virus was easily transmitted to 10 distinct leguminous hosts from 19 different host plants, including cowpea var. C-152. Other hosts tested for the presence of BCMV were found to be uninfected. Within plant species, the inoculation period required for symptom expression differed.

CONCLUSIONS

The results of the experiments conducted in this study demonstrated that BCMV may be readily transmitted to other host plants through mechanical sap inoculation and can display a range of symptoms however, the host plants incubation times for expressing different symptoms differed. The hosts that were found to be susceptible to BCMV after mechanical injection may act as a reservoir for the virus. Therefore, the data acquired for this study may help with the prognosis of diseases and the development of appropriate management approach.

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