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Biological and serological confirmation of host range of bean common mosaic virus (BCMV) infecting field bean

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Abstract
Bean common mosaic virus (BCMV) associated with field bean in GKVK (BCMV-Fb-GKVK) isolate was mechanically inoculated to different host plants. The host range studies were carried out to determine the biological relationship of BCMV-Fb-GKVK isolate with other host plants through mechanical sap inoculation method. Out of 22 host plants tested, BCMV was easily transferred to 11 different host plants. The incubation period taken for symptom expression was varied within the plant species. Soybean taken longer incubation period of 10-15 days for expression of symptoms like mosaic and veinal chlorosis. Whereas, lesser incubation period was noticed in Chenapodium (1-2 days) and tobacco (6-7 days). All plants were subjected to Double antibody sandwich- Enzyme linked immunosorbent assay (DAS-ELISA) to confirm the presence of BCMV. The results showed that, the host plants whichever exhibited different symptoms upon mechanical sap inoculation have reacted positively with the antibody specific to BCMV and produced bright yellow color thus, confirmed the presence of BCMV.

Keywords: Bean common mosaic virus, Mechanical inoculation, Incubation period and DAS-ELISA

Introduction
Field bean (*Lablab purpureus* L.) is one of the most ancient legume crops known for its food and fodder values (Shivakumar *et al.,* 2016) [20]. BCMV is believed to be originated from East or south Asia now it has distributed throughout the world wherever the legume crops are grown (Gibbs *et al.,* 2008 and El-kady *et al.,* 2014) [9, 7]. The virus was first reported in 1936 by McDonald and its identity was confirmed by Kulkarni in 1973. The BCMV has become serious problem for the bean cultivation wherever the crop is grown as it is reported to be transmitted through sap, seeds, pollens and aphids (Kennedy *et al.,* 1962; Trindade *et al.,* 1984; Puttaraju *et al.,* 2004; Kapil *et al.,* 2011) [11, 21, 17, 10].
In Karnataka, BCMV infecting field bean was first reported by Udayashankar *et al.* (2011) [22]. This virus is the type member of the genus *Potyvirus* belongs to family *Potyviridae*. The disease can cause significant yield losses of about 50 to 100 % in different host plants as reported by Drijfhout (1991) [6]. The plants infected with BCMV under natural field conditions is characterized by the systemic symptoms like mosaic, mottling, vein banding, twisting of leaf, uneven leaf lamina, vein clearing and puckering symptoms (Mangeni *et al.,* 2014) [12].
Emergence of plant viruses is a complex process driven by many direct and indirect interactions like ecological and genetic factors finally resulting in encountering of a new host, adapting to it and starts replicating in adopted host. Many of the viruses are entirely dependent upon their host's cells for their survival and replication. They have not only developed the ability to replicate in their host, but also to move between host cells and ultimately between hosts in order to persist (Fermin, 2018) [8]. The capacity of pathogens to infect a different host plants *i.e.,* to possess a large host range breadth (HRB), is tightly linked to their emergence propensity. Broader host ranges were observed for viruses with single-stranded genomes than double-stranded genome (almost exclusively RNA) and non-seed-transmitted viruses (Moury *et al.,* 2017) [16]. It is very clear that the causes of disease spread are mainly the result of direct and indirect interactions among which major focus is on host range studies of virus (McLeish *et al.,* 2019) [14].
The interrogation of host range of a virus is the key property of viruses, which clearly reflects the competence of the particular virus to infect other hosts naturally. To be a successful member of host range club of virus the susceptible host should support the life cycle of virus *i.e.,* series of tasks like unencapsidation, replication in the initial cell, movement from cell-cell and throughout the host system (Fermin, 2018) [8].

Several researchers had reported that mechanical sap inoculation is the best method to entrust BCMV to other host plants (Morris *et al.*, 2006; Bhadramurthy and Bhat, 2009 and El -Kady *et al.*, 2014) [15, 2, 7]. Solitarily mechanical sap inoculation is not much accurate method in understanding the host range of specific virus as it failed in distinguishing the symptoms produced by virus and abiotic factors as reported by Robert *et al.* (1991). Therefore, the blend of mechanical sap inoculation with other sensitive methods like ELISA could give accurate conviction of host range and strain identification (Bhadramurthy and Bhat, 2009) [2].

The knowledge and awareness about biological relationship like host range studies of specific virus is the important step to understand the etiology thus, helps in designing proper management strategy (Morris *et al.*, 2006) [15]. With this background the current investigation has been conducted to check out host range of BCMV infecting field bean and other host plants by employing mechanical sap inoculation and DAS-ELISA using BCMV specific antibody.

Material and Methods

Maintenance of BCMV inoculum: The field bean plants infected with BCMV were collected from the naturally infected field located at Zonal Agricultural Research Station, (ZARS), GKVK, Bengaluru. The collected samples were mechanically inoculated to field bean variety (HA-4) and the culture was maintained as a stock for further inoculation.

Mechanical inoculation: The culture of BCMV was maintained at Department of Plant Pathology, GKVK, Bengaluru and maintained stock culture was used for mechanical inoculation. The culture was crushed in pre-chilled pestle and mortar using phosphate buffer (0.1 M, p^H 7.0) and extracted sap was mechanically inoculated to two leaf stage healthy seedlings of different host plants dusted with celite (abrasive) and it was washed with water after 2-3 min to remove the excess inoculum. The ability of the BCMV to infect different host plants belonging to Chenapodiaceae, Solanaceae, Leguminaceae and Cucurbitaceae family were evaluated. Seeds of 22 different host plants were collected and sown in portrays, after 15 days the seedlings were transplanted to pots containing soil and sterilized coconut coir pith. In each plant species, 10 plants were inoculated and one set of un-inoculated plants were maintained as control. Inoculated plants were kept in the insect proof glass house and examined periodically for symptom expression.

Back inoculation test: The back inoculation test was carried out to confirm the etiology of virus. The infected leaves of plants were collected and back inoculated to propagation host mechanically to confirm viral etiology. The infected leaves of back inoculation test were used for serological assay *i.e.*, DAS-ELISA.

Serological assay: DAS-ELISA was employed for detection of virus infected field bean leaves after four weeks of inoculation. Polysterene plates were coated with anti-BCMV antibodies (LOEWE, Germany), diluted 1:200 in coating buffer and incubated for four hours at 37 ℃. Sap was extracted by grinding leaves in the extraction buffer in pestle and mortar and then centrifuged at 8000 rpm for 5 min. Exactly 200 µL of the extracted sap of sample was then added to the coated polysterene plate and incubated overnight at 4 ℃. Alkaline phosphatase (ALP) conjugated anti-BCMV antibody was added in 1:200 dilutions and incubated for four hours at 37 ℃, followed by incubation with p-nitrophenyl phosphate (AGDIA, India) at room temperature for 1 hr. The change in color at the end of test confirms the presence of BCMV and the absorbance values were measured on ELISA plate reader at 405 nm (Basavaraj, 2014) [1].

Result and Discussion

Out of 22 host plants tested, 11 plants showed different systemic symptoms *viz.*, mild mosaic, mosaic, mottling, leaf distortion, chlorotic lesion, leaf cupping, puckering, vein clearing, vein banding, vein netting and veinal chlorosis. There was a variation in incubation period taken for expression of symptom by different host plants. Soybean took longer incubation period (10-15 days) for expression of symptoms like mosaic and veinal chlorosis. Whereas, shorter incubation period was observed in Chenapodium (1-2 days) and tobacco (6-7 days) belonging to Chenapodiaceae family.

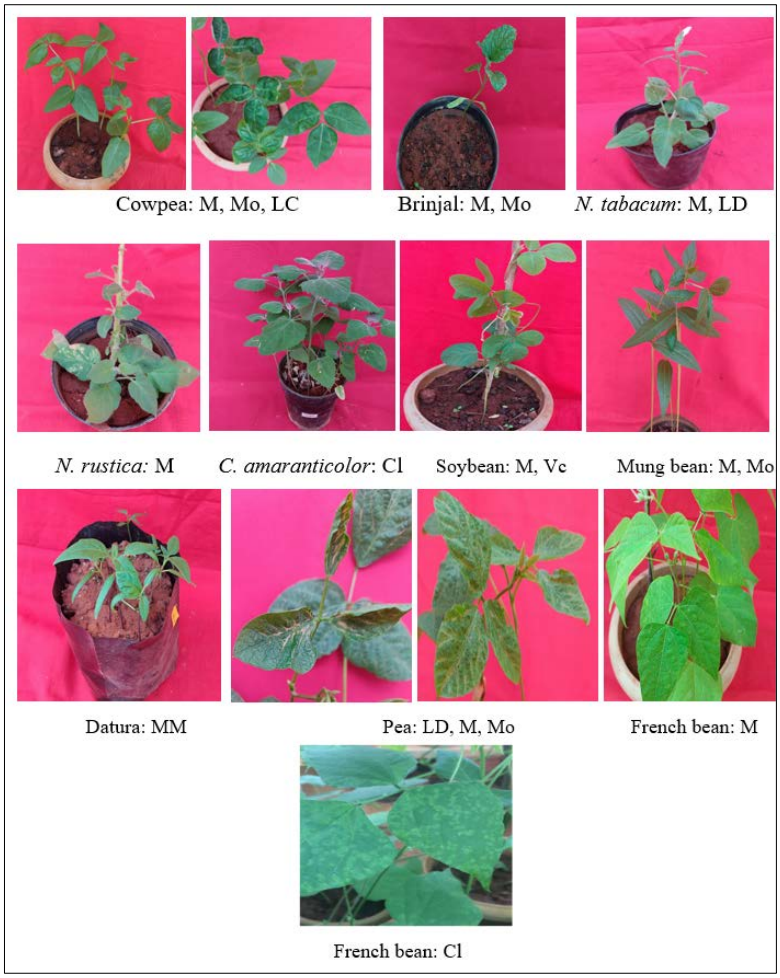
The host plants like datura and brinjal belonging to Solanaceae family taken incubation period of 10-12 days to express the symptoms like mild mosaic, mosaic and mottling, respectively. The host plants belonging to Leguminaceae family took incubation period of eight to twelve days for expression of different kind of symptoms *viz.*, mosaic and chlorotic lesion (French bean), mosaic and mottling (mung bean and pea). Similarly, mosaic, mottling and leaf cupping were noticed in cowpea. Whereas, mild mosaic, mosaic, mottling, leaf distortion, chlorotic lesion, leaf cupping, puckering, vein clearing, vein banding and vein netting symptoms were expressed by field bean within eight-ten days (Table 1 and Photo 1).

Table 1: Biological and serological detection of host range of BCMV-Fb-GKVK isolate

Sl. No.	Plant species inoculated	Family	No. of plants infected	Incubation period (dpi)	Symptoms exhibited	ELISA confirmation	OD value
1	Field bean (*Lablab purpureus* L.)	Leguminaceae	9	8-10	M, Mo, VC, VN, VB, P	+	1.82
2	French bean (*Phaseolus vulgaris* L.)		6	9-10	M, Cl	+	1.34
3	Mungbean (*Vigna radiata* L.)		6	8-12	M, Mo	+	1.08
4	Soybean (*Glycine max* (L.) Merr.)		6	10-15	M, Vc	+	1.02
5	Pegionpea (*Cajanus cajan* (L) Millsp.)		0	-	-	-	-
6	Chickpea (*Cicer arietinum*)		0	-	-	-	-
7	Moth bean (*Vigna aconitifolia*)		0	-	-	-	-
8	Lablab bean		0	-	-	-	-
9	Horsegram (*Macrotyloma uniflorum*)		0	-	-	-	-
10	Cluster bean (*Cyamopsis tetragonoloba*)		0	-	-	-	-
11	Pea (*Pisum sativum* L.)		5	10-11	M, Mo	+	1.06
12	Lima bean (*Phaseolus lunatus*)		0	-	-	-	-

13	Cowpea (*Vigna unguiculata* L.)		8	8-10	M, Mo, LC	+	1.68
14	Chilli (*Capsicum annum* L.)	Solanaceae	0	-	-	-	-
15	Datura (*Datura stramonium* L.)		5	10-12	MM	+	1.00
16	Brinjal (*Solanum melongena* L.)		8	10-12	M,Mo	+	1.02
17	Tomato (*Lycopersicon esculentum* Mill.)		0	-	-	-	-
18	*Chenapodium amaranticolor* L.	Chenapodiaceae	4	1-2	Cl	+	1.11
19	Tobacco (*Nicotiana tabaccum* L.)		7	6-7	M, LD	+	1.32
20	Tobacco (*Nicotiana rustica* L.)		6	6-7	M	+	1.30
21	Cucumber (*Cucumis sativus* L.)	Cucurbitaceae	0	-	-	-	-
22	Pumpkin (*Cucurbita moschata*)		0	-	-	-	-

*No of plants inoculated:10
MM- Mild mosaic, M- Mosaic, Mo- Mottling, LD- Leaf deformation, Cl- Chlorotic lesion, LC-Leaf cupping, P- Puckering, VC- Vein clearing, VB- Vein banding, VN- Vein netting, Vc- Veinal chlorosis
+ (positive reaction with BCMV specific antisera), dpi- days post inoculation`



M- Mosaic, Mo- Mottling, LD- Leaf distortion, Cl- Chlorotic lesion, LC-Leaf cupping, LD- Leaf deformation, Cl- Chlorotic lesion, Vc- Veinal chlorosis

Photo 1: Different plant species exhibiting varied symptoms upon mechanical inoculation with BCMV-Fb-GKVK isolate under greenhouse conditions

The plant species belonging to Cucurbitaceae family failed to produce any of the symptoms. The difference in incubation period taken by host plants for symptom expression might be due to poor response of respective host for multiplication as well as movement of virus and developmental stage of respective host plant (Zhang *et al.*, 2012) [23]. Even systemic invasion of virus in some hosts depends on genotype of host, age of the plant, temperature, strain of virus and type of host (Zitter and Murphy, 2009; Chellappan *et al.*, 2005) [24, 4]. The type of symptoms exhibited by virus depends on the interaction between resistance nature of host and pathogenicity genes of virus (Brunt *et al.*, 1996; Chung *et al.*, 2015) [3, 5]. BCMV failed to infect Pegionpea, Chickpea, moth bean, horse gram, cluster bean, lima bean, chilli, tomato, cucumber and pumpkin.

All these plants were subjected to DAS-ELISA to confirm the presence of BCMV. The results revealed that, the host plants whichever exhibited different symptoms have reacted positively with the antisera specific to BCMV and produced bright yellow color. The results presented in table 1 indicating that highest (1.82) and lowest (1.00) OD values were observed in field bean and datura, respectively. The OD values of infected plants were two to three times higher that of negative control (0.23) and buffer control (0.44). The obtained results are in agreement with the findings of Salgar *et al.* (2021) [19] carried out host range studies of BCMV by

sap inoculation method. The results showed that the BCMV virus was mainly confined to plants belonging to family Leguminaceae and symptoms observed on them included mosaic, mottling, blistering, puckering, necrosis, vein banding, reduction in pod size, overall stunting of plant, bending of leaf margins, downward curling of leaves as well as cupping of leaves.

Similarly, the results are also in harmony with studies conducted by Manjunatha *et al.* (2017) [13], studied the host range of BCMV by mechanically inoculating to different leguminous plants. Out of 19 host plants, including cowpea Var.C-152, the virus was easily transferred to 10 different leguminous hosts. Whereas, other hosts assessed for the presence of BCMV were seems to be uninfected. The incubation period taken for symptom expression was varied within plant species. Pole bean expressed symptom like mosaic after 15-18 days of incubation period whereas, common bean and rice bean took shorter incubation period of 7- 10 days. BCMV produced mosaic, chlorosis, leaf distortion, vein banding, puckering, vein netting, and vein clearing on cowpea(C-152). A mosaic symptom was observed in common bean, green gram, lima bean, yard long bean and rice bean, whereas, leaf distortion and leaf rolling was observed in pole bean, black gram and snap bean.

Conclusion

The experimental results of present study revealed that BCMV can be easily transferred to other host plants through mechanical sap inoculation and exhibited varied kind of symptoms but there was variation in incubation period taken by host plants to express different symptoms. The identified hosts *viz.*, hosts easily attacked by BCMV upon mechanical inoculation might acts as a reservoir of BCMV. Hence, the information produced in current study could help in prediction of emergence of disease and framing a proper management strategy.

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