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Identification of Potential Genotypes for Bean Common Mosaic Virus Resistance in Field Bean

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Authors Contribution

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Abstract

Evaluation of 50 genotypes was taken up under glasshouse conditions through mechanical sap inoculation method against bean common mosaic virus (BCMV). Among 50 genotypes screened none of the them were found immune to BCMV disease. Genotype (GLP-67-1) showed resistant (R) reaction, 26 genotypes showed moderately resistant (MR), 15 genotypes showed moderately susceptible (MS), five genotypes showed susceptible (S) and three genotypes showed highly susceptible (HS) reaction to BCMV. The genotypes were further screened through double antibody sandwich enzyme linked immune sorbent assay (DAS-ELISA) and the results revealed that genotypes which had exhibited bean common mosaic disease associated symptoms in glasshouse condition showed strong positive reaction to specific antibody of BCMV by producing bright yellow color.

Keywords : Bean common mosaic virus, Resistant, Screening, Susceptible, DAS-ELISA

Field bean (*Lablab purpureus* L.), also known as faba beans or lablab beans, is one of the most ancient legume crops known for its food and fodder values (Shivakumar *et al.,* 2016). It is the third most important leguminous crop after soybean (*Glycine max* L.) and peas (*Pisum sativum* L.) (Singh *et al.,* 2013 and Paunina *et al.,* 2018). It is a multiutility and multi-beneficial leguminous crop, extensively grown for vegetable, pulse, fodder, green manure, cover crop, medicine and ornamental purpose. Despite of its importance as a multi-purpose crop, it can withstand drought better than cowpea (Ramesh and Byregowda, 2016). Karnataka contributes about 90 per cent of both area and production of field bean in India (Praneetha *et al.,* 2022).

Field bean has been exposed to variety of diseases caused by fungi, bacteria, viruses and nematodes with losses estimating millions of rupees annually. Among the viral diseases, dolichos yellow mosaic, dolichos enation, leaf roll, bean common mosaic and bean common mosaic necrosis (Mwaipopo *et al.* 2017) diseases have been reported in India (Capoor and Verma, 1950).

In Karnataka, BCMV infecting field bean was first reported by Udayashankar *et al.* (2011). This virus is the type member of the genus *Potyvirus* belongs to family *Potyviridae*. This disease causes significant yield losses (50 to 100 %) in different host crop plants (Drijfhout, 1991). The plants infected with BCMV is characterized by mosaic, mottling, vein banding twisting of leaf, uneven leaf lamina, vein clearing and puckering symptoms (Mangeni *et al.,* 2014). Management of viruses is generally a difficult task since the viruses are systemic and contagious in nature. Hence, identification of resistant genotypes has been considered as the most efficient approach against plant viruses (Tewari and Ramanujam, 1994). Keeping these aspects in view, an attempt has been made to identify the resistant genotypes against BCMV.

Material and Methods

The screening was conducted under glasshouse conditions at the Department of Plant Pathology, UAS, GKVK, Bengaluru by using completely randomized design (CRD) with three replications and five plants in each replication. Fifty field bean genotypes were screened for resistance against BCMV. The genotypes were collected from All India National Project (AINP) on Arid legumes, Zonal Agricultural Research Station, (ZARS), GKVK, Bengaluru.

Maintenance of BCMV culture : The field bean plants showing characteristic symptoms of BCMV *viz.,* mosaic, mottling, vein banding, vein netting, vein clearing and puckering (Fig. 1) were collected from the naturally infected field at the ZARS, GKVK, Bengaluru.

The infected leaf sample was crushed using phosphate buffer (0.1 M, p^H 7.0) and mechanically inoculated to two leaf stage old healthy susceptible genotype of field bean *i.e.,* GL-161. The inoculated plants were kept in insect proof cage for symptom expression under glasshouse conditions. These plants were used as stock culture and maintained continuously by inoculating to healthy field bean plants at regular intervals. The maintained stock culture was used as a source of inoculum for screening. Fifteen seeds of each genotypes were sown in pro-trays which contained coco-peat with nutrient mixture and maintained upto two leaf stage. Then the seedlings were transplanted to pots containing sterilized soil for inoculation studies. After transplanting, the seedlings were inoculated with the BCMV.

Mechanical inoculation of BCMV : The field bean plants at two leaf stage just above the cotyledon leaves were sap inoculated mechanically as described by Ashfaq *et al.* (2010). After inoculation, plants were jet sprayed with water to remove excess inoculum and abrasive (Carborandum). Field bean genotype GL-161 was used as a susceptible check throughout the screening studies.

Assessment of disease severity : Thirty days after inoculation, disease severity scores were noted according to the scale given by Diwakar and Mali, 1976 (Table 1). However, little little modification in the scale was made based on the different symptoms exhibited by the genotypes. The per cent disease index (PDI) was calculated using the formula given by Wheeler (1969). Based on the PDI values obtained, the genotypes were classified into five categories (Table 1).

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{maximum disease grade}} \times 100$$

Serological Assay

DAS-ELISA was employed for investigation of virus infected field bean leaves after four weeks of inoculation. Polysterene plates were coated with anti-BCMV antibodies (LOEWE, Germany), diluted

A : Mosaic and Vein netting B : Mottling and puckering C. Vein banding and vein clearing

Fig. 1: Naturally infected field bean plants showing peculiar symptoms of BCMV

TABLE 1
Disease reaction scale (0-5) (Diwakar and Mali, 1976)

Scale	Description	Type of symptoms	Category
0	No disease symptoms	No symptoms	Immune
1	1-5 per cent of plants showing symptoms	Only mild mosaic	Resistant
2	5-15 per cent of plants showing symptoms	Mild mosaic or Mosaic or Mottling	Moderately resistant
3	15-25 per cent of plants showing symptoms	Mild mosaic or Mosaic and Mottling	Moderately susceptible
4	25-50 per cent of plants showing symptoms	Mild mosaic or Mosaic, Mottling or Puckering and vein netting	Susceptible
5	>50 per cent of plants showing symptoms	Mild mosaic, Mosaic, Mottling, Vein clearing, Vein banding, Puckering and Vein netting	Highly susceptible

1:200 in coating buffer and incubated for four hours at 37 °C. 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 °C. Alkaline phosphatase (ALP) conjugated anti-BCMV antibody was added in 1:200 dilutions and incubated for four hours at 37 °C 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).

RESULTS AND DISCUSSION

A total of 50 genotypes of field bean were screened for resistance against BCMV in glasshouse conditions. A perusal of the data in Table 2 and Fig. 3 revealed that different type of reactions were reflected by different genotypes in the form of severity of disease expressed as per cent. The results indicated that highest PDI (88.89) was recorded in susceptible check (GL-161) and lowest PDI was noticed in GLP-67-1 (2.42). Out of 50 genotypes screened, none of them showed immune reaction, only one genotype *i.e.,* GLP-67-1 exhibited resistant (2 PDI) reaction and 26 genotypes *viz.,* GLP-15, GLP-77, GLP-75, GLP-10-1, GL-145, GL-58, GL-46, GL-109, GL-266, GL-251, GL-569, GL-157, GL-65, GL-83, GL-7, GL-140, GL-110, GLB-25, GLB-22, GLB-18, GLB-11, GLB-12, GLB-17, GLB-14, GLB-8 and GLB-7 showed moderately resistant (52 PDI) reaction. Fifteen genotypes *viz.,* GLP-9, GL-156, GLP-14, GL-139, GL-119, GL-529, GL-131, GLB-24, GLB-19, GLB-15, GLB-16, GLB-6, GLB-9, GLB-13 and GLB-3 showed moderately susceptible (30 PDI) reaction, five genotypes *viz.,* GLB-1, GLB-4, HA-3, GLB-23 and GL-13 showed susceptible reaction (10 PDI) and three genotypes *viz.,* GLB-2, GLB-5 and GL-161 exhibited highly susceptible (6 PDI) reaction (Fig. 3).

Screened genotypes showed different kinds of characteristic symptoms *viz.,* mild mosaic, mosaic, mottling, vein clearing, vein banding, vein netting and puckering upon mechanical inoculation (Fig. 2 and Fig. 4). For further confirmation, all genotypes were screened through DAS-ELISA with aid of anti-BCMV specific antibody.

The results of DAS-ELISA revealed that the absorbance value of resistant genotype was 0.42, whereas, moderately resistant and moderately susceptible genotypes absorbance value ranged from 0.44-0.63 and 0.73-1.15, respectively. The absorbance value for susceptible and highly susceptible genotypes were ranged from 1.13-1.58 and 1.6-1.89, respectively.

These genotypes were further categorized into different groups *viz.,* immune (0), resistant (1), moderately resistant (2), moderately susceptible (3), susceptible (4) and highly susceptible (5) based on

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TABLE 2

Reaction of field bean genotypes to BCMV infection under glasshouse conditions

Geno-types	10 DPI	15 DPI	30 DPI	45 DPI	Disease score	Disease reaction	Severity	OD value @ 405nm
GLB-1	MM	MM, Mo	MM, Mo,VN	MM, Mo,VN	4	S	44.44	1.58
GLB-2	MM	MM,M,Mo	MM,M,Mo,VB	MM,M,Mo,VB, VC	5	HS	83.28	1.79
GLB-3	MM	Mo, MM	Mo, MM	Mo, MM	3	MS	18.28	1.09
GLB-4	M	M,P	M, P,VN	M, P,VN	4	S	41.26	1.49
GLB-5	MM	MM,M, Mo Mo,VN	MM, M, Mo,VN,VB	MM,M, Mo,VN, VB	5	HS	78.63	1.62
GLB-6	MM	MM,M	MM,M	MM,M	3	MS	17.23	0.96
GLB-7	MM	MM,Mo	MM,Mo	MM,Mo	2	MR	10.21	0.52
GLB-8	M	M	M	M	2	MR	8.38	0.46
GLB-9	M	M,Mo	M,Mo	M,Mo	3	MS	19.85	0.11
GLB-11	MM	MM,Mo	MM,Mo	MM,Mo	2	MR	9.36	0.47
GLB-12	MM	MM, M, Mo	MM, M, Mo	MM, M, Mo	2	MR	13.69	0.63
GLB-13	MM	MM, Mo	MM, Mo	MM, Mo	3	MS	19.12	1.00
GLB-14	NS	MM,M	MM,M	MM,M	2	MR	10.57	0.54
GLB-15	NS	M	M, Mo	M, Mo	3	MS	20.67	1.12
GLB-16	NS	MM, M	MM, M	MM, M	3	MS	17.83	0.98
GLB-17	NS	MM, Mo	MM, Mo	MM, Mo	2	MR	9.57	0.51
GLB-18	NS	MM, Mo	MM, Mo	MM, Mo	2	MR	10.13	0.59
GLB-19	NS	M, MM	M, MM	M, MM	3	MS	18.32	0.99
HA-3	MM	Mo, MM	Mo, MM	Mo, MM	4	S	36.96	1.38
GLB-22	NS	MM,M	MM,M	MM,M	2	MR	9.54	0.52
GLB-23	NS	M, Mo	M, Mo, VN	M, Mo, VN	4	S	39.12	1.86
GLB-24	NS	M, Mo	M, Mo	M, Mo	3	MS	20.61	1.08
GLB-25	NS	MM,M	MM, M	MM, M	2	MR	8.57	0.49
GL-136	NS	MM,M	MM,M	MM,M	4	S	35.33	1.13
GL-131	NS	MM, Mo	MM, Mo	MM, Mo	3	MS	18.36	0.93
GL-83	MM	MM, Mo	MM, Mo	MM, Mo	2	MR	11.24	0.60
GL-7	MM	MM, M	MM, M	MM, M	2	MR	10.21	0.53
GL-140	NS	MM,Mo	MM, Mo	MM, Mo	2	MR	12.63	0.61
GL-110	MM	MM, M	MM, M	MM, M	2	MR	9.56	0.53
GL-119	MM	MM,M	MM, M	MM, M	3	MS	19.96	0.98
GL-529	NS	MM,M	MM,M	MM,M	3	MS	19.45	0.96
GL-109	NS	M	M	M	2	MR	7.36	0.46
GL-266	MM	MM, Mo	MM, Mo	MM, Mo	2	MR	9.65	0.49
GL-251	M	M, Mo	M, Mo	M, Mo	2	MR	12.96	0.63

Continued.....

Geno-types	10 DPI	15 DPI	30 DPI	45 DPI	Disease score	Disease reaction	Severity	OD value @ 405nm
GL-145	M	M	M	M	2	MR	7.36	0.44
GL-58	NS	MM,M	MM,M	MM,M	2	MR	9.65	0.48
GL-46	NS	M, Mo	M, Mo	M, Mo	2	MR	10.21	0.51
GL-139	NS	MM	MM	MM	3	MS	17.24	0.54
GL-161	NS	MM,M, Mo, VB, VC	MM, M, Mo, VB,VC, P	MM,M, Mo, VB,VC, P	5	HS	88.89	1.89
GL-156	NS	M	M,Mo	M,Mo	3	MS	21.96	1.13
GLP-10-1	NS	M,MM	M,MM	M,MM	2	MR	9.24	0.48
GLP-67-1	NS	MM	MM	MM	1	R	2.42	0.42
GLP-9	M	M,MM	M,MM	M,MM	3	MS	15.64	0.93
GLP-14	M	M, Mo	M, Mo	M, Mo	3	MS	21.21	1.10
GLP-15	MM	MM, M	MM, M	MM, M	2	MR	10.21	0.52
GLP-77	NS	M	M	M	2	MR	8.11	0.48
GLP-75	MM	MM,M	MM,M	MM,M	2	MR	9.56	0.53

MM- Mild Mosaic, M- Mosaic, Mo- Mottling, P- Puckering, VC- Vein Clearing, VB- Vein Banding, VN- Vein Netting, DPI- Days Post Inoculation, R- Resistant, MR- Moderately Resistant, MS- Moderately Susceptible, S- Susceptible, HS- Highly Susceptible

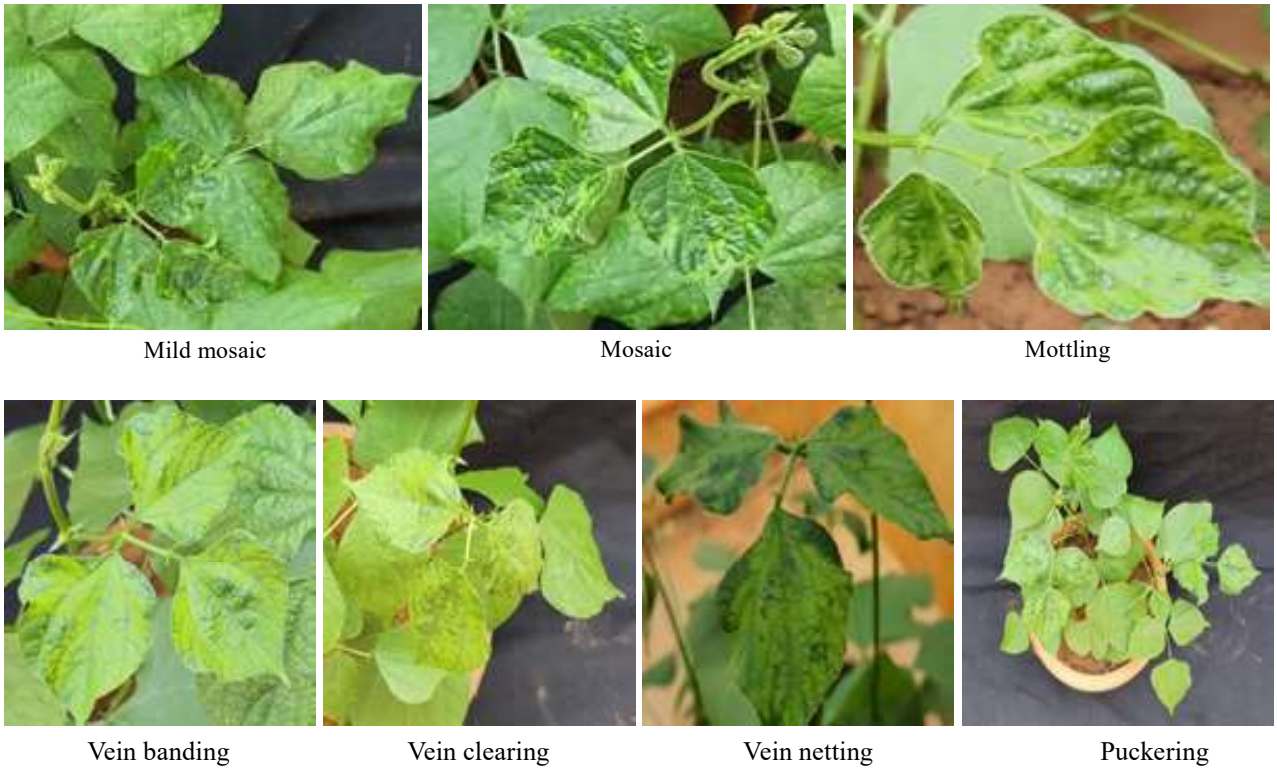

Fig. 2: Field bean leaves showing different kinds of BCMV symptoms upon mechanical inoculation

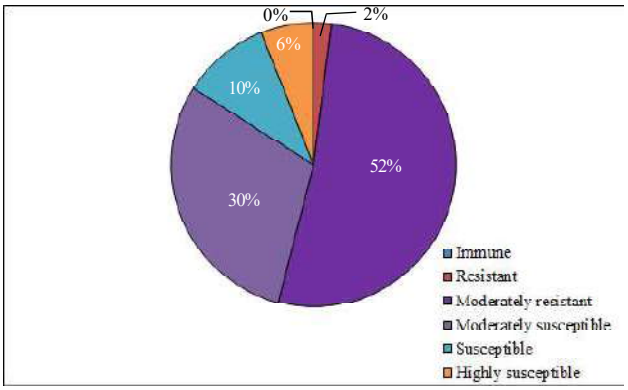

Fig. 3: Grouping of field bean genotypes based on BCMV disease reaction under glasshouse

Fig. 4: Field bean genotypes exhibiting different kind of symptoms upon artificial inoculation

the PDI value obtained by respective genotypes (Table 3).

The results are in agreement with that of screening experiment conducted by Renuka (2014), in which 75 field bean genotypes were screened against BCMV under glasshouse conditions at UAS, GKVK, Bengaluru. Among 75 field bean genotypes were screened, only one genotype (Kadale avare) showed moderately resistant (MR) reaction, 22 genotypes showed susceptible (S) reaction and remaining 52 genotypes showed highly susceptible (HS) reaction. The results obtained are also in conformity with Manjunatha *et al.* (2021), where 350 common bean genotypes were screened under natural conditions and artificial inoculation method during 2016-2017, 2017-2018 and 2018-2019. Eleven genotypes *viz.,* EC400445, EC400414, ET400414, ET4515B, EC540173, IC360831, BD9116291, EC31084, EC541703, ET8409, IC340947 and IC356024 were found resistant.

Use of resistant genotype is regarded as a durable method for controlling plant diseases mainly the viral diseases. By this background, screening of 50 genotypes against BCMV at GKVK, Bengaluru was undertaken. From this study it can be revealed that field bean genotypes showing resistant, moderately resistant reaction to BCMV could serve as potential donor of resistance in breeding programme. The major drawback in evaluation of genotypes is that some genotypes which had shown resistant in one location could turn out to be susceptible in location because of environmental impact on genotype and environmental-genotype interaction need to studied for durable resistance in future.

TABLE 3

Grouping of field bean genotypes based on reaction to BCMV disease under glasshouse conditions

Scale	Category	Genotypes
0	Immune	Nil
1	Resistant	GLP-67-1
2	Moderately resistant	GLP-15, GLP-77, GLP-75, GLP-10-1, GL-145, GL-58, GL-46, GL-109, GL-266, GL-251, GL-569, GL-157, GL-65, GL-83, GL-7, GL-140, GL 110, GLB-25, GLB-22, GLB-18, GLB-11, GLB-12, GLB-17, GLB-14, GLB-8, GLB-7
3	Moderately susceptible	GLP-9, GL-156, GLP-14, GL-139, GL-119, GL-529, GL-131, GLB-24, GLB-19, GLB-15, GLB-16, GLB-6, GLB-9, GLB-13, GLB-3
4	Susceptible	GLB-1, GLB-4, HA-3, GLB-23, GL-13
5	Highly susceptible	GLB-2, GLB-5, GL-161

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