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Screening of Soybean Genotypes against Cowpea Mild Mottle Virus Infection

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ABSTRACT: Cowpea mild mottle virus (CPMMV), a whitefly and seed transmitted carla virus, is an emerging soybean disease in India and many tropical and subtropical regions of Africa and Asia. This virus causes significant yield and quality losses under field conditions. The utilization of resistant varieties is the best strategy to manage the losses caused by the CPMMV. Keeping this in mind, a field experiment was conducted at ICAR-IARI, New Delhi, situated at 28.6° N latitude, 77.16° E longitude at an elevation of 228.61 m above the mean sea level. The present study was conducted to identify the soybean genotypes resistant against cowpea mild mottle virus infection. Soybean genotypes with varied symptoms; mild mosaic, systemic mottling, stunting and leaf deformation were observed under field conditions. The CPMMV disease incidence in the soybean field varied from 10-85% during *Kharif* 2019. The field screening study identified fifty soybean genotypes as resistant to moderately resistant, while 40 genotypes showed susceptible reactions. These fifty genotypes were phenotyped using sap inoculation under controlled conditions. The glasshouse study showed that nine (18%) soybean genotypes showed a moderately-resistant reaction, nine genotypes were moderately susceptible, thirteen genotypes showed a susceptible reaction and 19 genotypes were highly susceptible against CPMMV infection. The current study would help in the utilization of identified genotypes in soybean resistance breeding to develop CPMMV resistant varieties and study host-pathogen interaction.

Keywords: CPMMV, mottle, genotypes, resistance, mechanical inoculation.

INTRODUCTION

Cowpea mild mottle virus (CPMMV), single positive strand RNA virus, a member of the genus Carlavirus (family: *Betaflexiviridae*), was first reported from cowpea (*Vigna unguiculata*) in Ghana (Brunt and Kenten 1973; Yang *et al*., 2022). Consequently, it was described from several plant species grown in tropical and subtropical countries in Africa and Asia (Zanardo and Carvalho 2017; Wei *et al*., 2020). The CPMMV produces local lesions on *Chenopodium amaranticolor* with varied severity depending on the isolate used for infection (Naidu *et al.,* 1998). The CPMMV is able to experimentally infect various host species within the plant families of *Asteraceae, Aizoaceae, Amaranthaceae, Cucurbitaceae, Chenopodiaceae, Pedaliaceae, Solanaceae,* etc. (Mansour *et al*., 1998; Pardina *et al*., 2004). The geographical range of CPMMV is very wide, and it has been documented inmore than 30 countries spread across the world (Zanardo and Carvalho 2017). The CPMMV induces variable symptoms, which include severe stem necrosis, systemic mottling, bud blight and stunting (Jossey, 2013; Wei *et al*., 2021). CPMMV is also reported to be symptomless in a few soybean varieties, and it also impacts other legumes like mungbean and peanut, nevertheless, the impact of CPMMV is less in soybean as compared to other crops (Zanardo and Carvalho 2017).

CPMMV has emerged as a major threat to soybean crop production in India and worldwide. It is known to cause soybean stem necrosis disease. As the name suggests, the disease caused by this virus is highly virulent, as it leads to the death of plants due to its systemic nature (Zanardo *et al*., 2014). CPMMV is unique among other carlaviruses as it is transmitted by the whitefly (*Bemisia tabaci*) in a non-persistent manner rather than aphids (Naidu *et al*., 1998). The whitefly is reported as an important polyphagous invasive pest that plays a role in the evolution of new virulent strains of CPMMV.

CPMMV is afilamentous rod shaped particle, measuring about 650 × 15 nm in size (Almeida *et al*., 2005). The CPMMV genome consists of a single-stranded linear RNA molecule (approximately 8.12 kb). It consisted of six putative ORFs; ORF5 (6833–7696 nt) and ORF6 (7702–8007 nt) encode coat protein (CP; 32.3 kDa) and nucleic acid–binding protein (NABP; 11.7 kDa), respectively (Menzel *et al*., 2010). The CPMMV occurrence in groundnut was reported from India (Mali *et al*., 1987). The transmission of CPMMV by *B. tabaci* in groundnut and soybean in India was demonstrated (Muniyappa and Reddy 1983; Mali *et al*., 1987). Two CPMMV strains (severe and mild strains) from groundnut were molecularly characterized based on the sequencing of the 3′-terminal region (Naidu *et al*., 1998). In recent years, systemic mottling, mosaic and deformed leaves caused by CPMMV have become a major issue, especially in soybean, cowpea, groundnut, brinjal, mungbean, broad bean, common bean, and tomato (Almeida *et al*., 2005; Menzel *et al*., 2010; Brito *et al*., 2012; Chang *et al*., 2013; Lamas *et al*., 2017). Soybean crops showing high disease incidence with mosaic, systemic mottling, stunting and chlorotic symptoms had been previously reported from New Delhi (Yadav *et al*., 2013). The mosaic disease caused by CPMMV leads to significant yield losses and deteriorates seed quality under severe infection (Yadav *et al*., 2013). Vector control is not only costly but also an environmentally hazardous task.

Additionally, the resistance source identified must have shown durability against both virus and vectors (Zanardo and Carvalho 2017). The deployment and exploration of CPMMV tolerant cultivars could be a better option to manage CPMMV infection (Mituti and Almeida 2006). Several workers have identified soybean genotypes/cultivars showing resistance against CPMMV infection in several countries, including India (Almeida, 2008; Suryanto *et al*., 2014; Arias *et al*., 2015; Cheruku *et al*., 2017; Oliveira *et al*., 2018). The utilization of resistant varieties is the best option to manage the losses caused by plant pathogens (Yadav *et al*., 2017; Susan *et al*., 2018). Therefore, the present study was designed to identify the resistant sources from diverse soybean genotypes that could be utilized as donors and study host pathogen interactions.

MATERIALS AND METHODS

A. Disease survey, screening and disease assessment of soybean genotypes under field conditions

A disease survey was made in soybean crops grown in the experimental farm of ICAR- Indian Agricultural Research Institute (IARI), New Delhi, during the *Kharif* season of 2019. The disease incidence was recorded 45 days after sowing to record symptoms and the disease incidence of CPMMV.

The disease screening was conducted in the experimental farm of ICAR-IARI, New Delhi, India, situated at 28.6° N latitude, 77.16° E longitude at an elevation of 228.61 m above the mean sea level. A set of 100 soybean genotypes were obtained and grown during the *kharif*, season of 2019, and screened under open field conditions. A standard package of practices was followed to raise the soybean crop. The essential cultural practices were performed for the raising of the soybean crop. The disease reactions for CPMMV were recorded 45 days after sowing (DAS). The genotypes were later grouped into different categories, from resistant to highly susceptible, as per the disease scale described previously (Arif and Hassan 2002) (Table 1). The disease was recorded twice, and higher values were taken as final readings. Based on disease spectrum data, a set of 50 genotypes was further selected for controlled screening under insect proof glasshouse conditions.

Table 1: Disease scoring scale for CPMMV disease severity.

Rating Scale	Description (visual observation)	Reaction
1	No symptoms (apparently healthy plant)	Resistant
2	Slightly mosaic leaves (10 – 30 %)	Moderately resistant
3	Mosaic (31 – 50 %) and leaf distortion	Moderately susceptible
4	Severe mosaic (51 – 70 %), leaf distortion and stunting	Susceptible
5	Severe mosaic (>70 %), stunting and death of plants	Highly susceptible

B. Virus isolates and plant inoculation

The soybean leaves showing characteristic symptoms in the field were selected and transmitted to *Chenopodium quinoa*(the local lesion host) through sap inoculation. Then, a local lesion developed on the local lesion host was further transferred to the propagation host. The JS335 soybean cultivar, grown under insect proof conditions, was used as a propagation host. Further, the CPMMV infection was confirmed through an enzyme linked immuno-sorbent assay (ELISA) and PCR assay. The Delhi isolate was used for screening the selected genotypes of soybean under insect proof controlled conditions.

C. Controlled screening of soybean genotypes and disease assessment

Seeds of different soybean genotypes were sown in 12 inch pots filled with pot mixture (soil and compost in a 2:1 proportion). Insect proof cages were used to cover each pot. Each genotype was sown in 10 pots with 3 seeds per pot in a completely randomized manner. Then, the soybean plant attaining the trifoliate stage was used for sap inoculation based screening. Fresh soybean leaves were obtained from the JS335 cultivar and homogenized in a chilled mortar and pestle using inoculation buffer (0.1 M potassium phosphate) supplemented with 0.2% mercaptoethanol to yield a finely ground virus suspension. The test plants were first dusted with carborundum powder (amild abrasive) to aid in sap transmission and the inoculation was performed on the first trifoliate leaf of the test plants with the help of sterile cotton swaps. After sap transmission, the excess inoculum was removed by spraying water with the help of a sprayer. Mock inoculations were performed for each entry using a buffer solution. The inoculated plants were kept in an insect-proof greenhouse. Regular observation was done, and symptoms were recorded for each entry. The soybean genotypes were recorded as resistant or susceptible based on the disease scale described previously (Arif and Hassan 2002).

RESULT AND DISCUSSION

Recently, a whitefly transmitted, carlavirus named CPMMV has become a major issue in soybean products, affecting not only yield but also quality. The use of resistant lines would help in the better management of viral infections as they are cost effective and part of a sustainable strategy. The disease incidence was calculated from the ICAR-IARI, soybean experimental field. The symptoms associated with mosaic disease caused by CPMMV included systemic mottling, mosaic, several types of leaf deformation; crinkle, blistering, and leaf defoliation with stunting of the plant (Fig. 1). Similarly, CPMMV induces symptoms like chlorosis, mottling and mild mosaic in sap inoculated soybean plants (da Silva *et al*., (2020). The severity of the mosaic disease varied from one genotype to other genotypes. The disease incidence of CPMMV varied from 10-85%. A high CPMMV disease incidence (25.1-71.0%) was also observed in the *Kharif* season in New Delhi (Yadav *et al*., 2013). Similarly, 70% disease incidence was observed in the sap-inoculated soybean plants (da Silva *et al*., 2020). The high disease was observed due to the secondary spread of CPMMV through whiteflies.

Fig. 1. Reaction of soybean genotypes to CPMMV infection in the field conditions.

A. Evaluation of the soybean genotypes under field and controlled conditions against CPMMV

The identified resistance source should be durable and operative against both viral strains and vectors. In the present study, a set of 100 soybean genotypes were screened for CPMMV resistance under field conditions. The symptoms developed 35-40 days after sowing in the field. Based on the disease score, against CPMMV infection under field conditions, among the 90 genotypes tested, 50 genotypes showed a resistant to moderate reaction against CPMMV infection, while 40 soybean genotypes showed a susceptible reaction. These fifty soybean genotypes showing resistant to a moderately resistant disease spectrum were selected for further screening under insect proof conditions. In this study, JS335 was used as a susceptible control for phenotypic analysis against CPMMV. Among, 50 soybean genotypes, screened, under insect proof-conditions, eleven (22%) soybean genotypes showed a moderate-resistant reaction (score; 2), nine genotypes (18%) exhibited a moderately susceptible reaction(score of 3), thirteen genotypes (26%) exhibited a susceptible reaction (score of 4), and seventeen (34%) genotypes were highly susceptible (score of 5) (Fig. 2). The ratio of resistant genotypes to the total soybean genotypes was found to be 11/50 (22%). The screening score varied from 2 to 5 (Table 2). The lack of complete resistance might be due to a lack of resistance genes in the soybean genotypes tested. Yadav *et al.* (2013) screened twenty seven soybean cultivars under controlled conditions, but none was found to be resistant against CPMMV infection. In another study, one hundred thirty-three soybean cultivars were phenotyped against CPMMV infection and two cultivars (DS12-5 and SL958) showed resistance reactions against CPMMV infection (Cheruku *et al*., 2017). Further, utilization of tolerant soybean cultivars could be a good alternative to manage the CPMMV infection (Mituti and Almeida 2006). It was observed that, the tolerant varieties were able to recover faster after CPMMV infection as compared to susceptible cultivars in terms of infection, reduced virustiter and further spread of CPMMV.

Fig. 2. Symptom induced by CPMMV in soybean genotypes under controlled conditions.

Therefore, the tolerant cultivar might help in reducing the secondary spread of CPMMV in the field (Mituti and Almeida 2006). An alternative, economical and effective way to manage this emerging virus problem is to develop improved soybean cultivars with CPMMV resistance. Further, planting these resistant soybean varieties would help in increasing soybean production (Suryanto *et al*., 2014).

Further, Almeida (2008) identified eight resistant soybean cultivars against CPMMV infection among 170 soybean cultivars tested through sap inoculation. In another study, fifty soybean cultivars were screened and 92% showed tolerance to CPMMV while only8% were susceptible (Brizola *et al*., 2015). Suryanto *et al*. (2014) studied the number of *R*-genes associated with CPMMV resistance and its inheritance pattern. They used two resistant soybean genotypes to study the inheritance pattern and identified additive mechanism. These studies present the ability of CPMMV to infect multiple soybean cultivars with low levels of CPMMV resistance in soybean cultivars. However, the identified resistant or tolerant soybean cultivars would help in a soybean resistance breeding program to thwart and contain the CPMMV infection in farmer's fields.

Table 2: Disease reactions of soybean genotypes against CPMMV infection under insect proof glass house conditions.

Sr. No.	Disease Reaction	No. of lines	Name of genotypes
1.	Resistant	0	-
2.	M-Resistant	11	EC-472016, EC-472066, EC-472120, EC-472132, EC-472145, EC-472150, EC-472157, EC-472162, and EC-472191
3.	Moderately Susceptible	9	EC-472001, EC-472036, EC-472126, EC-472137, EC-472141, EC-472154, EC-472183, EC-472221, and EC-472217
4.	Susceptible	13	EC-471991, EC-472121, EC-472119, EC-472134, EC-472139. EC-472143, EC-472149, EC-472161, EC-472184, EC-472199, EC-472210, EC-472216, and EC-472222
5.	Highly susceptible	17	EC-471979, EC-471998, EC-471999, EC-472024, EC-472063, EC-472064, EC-472079, EC-472100, EC-472128, EC-472130, EC-472136, EC-472195, EC-472196, EC-472202, EC-472214, EC-472219, EC-472220, EC-472197, and EC-472203
	Total	**50**	

CONCLUSIONS

Field screening complemented with controlled glasshouse screening of soybean genotypes would help in identification of resistant genotypes against CPMMV infection. This would result in their rational use through their utilization in soybean resistant breeding programs, identification of novel gene(s)/allele(s) and study of host pathogen interaction at molecular level.

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Conflict of Interest. None.

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