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Symptomological Studies and Serodiagnosis of Viruses Associated with Soybean Floral Bud Distortion in Vidarbha Region of Maharashtra, India

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A B S T R A C T

Soybean (*Glycine max* L.) is one of the most important crops in the world and serves as a principal dietary food and oil source. It is the major cash crop of central zone of India. Due to the occurrence of no-podding syndrome (NPS) or floral bud distortion (FBD), more than 80% yield losses was occurred. Average FBD incidence ranged from 8.0 to 14.6% in different parts of the states. The symptomatic plants produced distorted flowers and either no or deformed pods and remained green after maturity. Also infected plants showed thicker stem and shorter internodes as compared to the asymptomatic plants. The viruses associated with FBD were detected and diagnosed applying the serological method such as Direct Antigen Coating- Enzyme Linked Immuno Sorbent Assay (DAC-ELISA). Soybean samples with FBD analyzed serologically by DAC-ELISA showed positive reaction and confirmed the presence of *Tobacco streak virus* (TSV) and *Groundnut bud necrosis virus* (GBNV).

Introduction

Soybean is the world's most important legume in terms of production and trade due to its high content of protein (40%) and oil (20%). In India, Madhya Pradesh, Maharashtra and Rajasthan are the major growing states accounting for~ 95% of world production (SOPA, 2017). Soybean is the major cash crop of central zone of India. A disease characterized as FBD of soybean causing more than 80% yield losses in Maharashtra, India. According to the preliminary estimates of the Department of Agriculture, Government of Madhya Pradesh, the area affected with NPS as about 5,000 ha in Indore alone followed by 37 ha in Vidarbha region, Maharshtra (Jadhav *et al.*, 2013). In recent year crop losses in pulse due to infection by GBNV has increased and 52% disease incidence and 92% yield losses in certain cultivar of mungbean (*Vigna radiata*) (Biswas *et al.*, 2009). A low familiarity with symptoms and weak diagnostic capacity in many countries hampers detection and effective control. Due to complex nature of the virus-plant-environment interactions, NPS management and control in soybean requires fast and accurate detection system.

One of the major constrains in pulse production are pathogens and viruses are among the most important groups of plant pathogens affecting pulse production worldwide. The proper identification of symptoms and serological identity of the soybean isolate causing FBD in India was not addressed. Thus, the present study was aimed to determine the symptomological and serological characterization of viruses associated with soybean isolate from this region. Serology is mostly sufficient for the proper identification of plant viruses and ELISA remains among the major developments that have taken place in plant virus detection.

Materials and Methods

The soybean plants showing symptoms of FBD (deformed pod formation), leaf necrosis and chlorotic lesions were collected from five different location of Vidarbha region, Maharashtra (i.e., Akola, Amravati, Buldhana, Nandura and Wanirambhapur). The samples were kept at -80°C in plastic bags in deep freeze with labels indicating date of collection and the location from where it was collected. Both field and laboratory studied were done with collected samples.

Symptomological study

Field survey was conducted for the calendar year from 2012-2014 for sample collection and to study the symptomatic plants from different regions.

DAC-ELISA

The possible ways to detect plant viruses, serological techniques are frequently favoured because of their specificity, speed and the scope they provide for standardization. The samples collected from different locations of Vidarbha region, Maharashtra, were sent to IARI (Indian Agricultural Research Institute), New Delhi for DAC-ELISA testing for positive association of TSV and GBNV. Antisera to GBNV and TSV was obtained from Plant Virology Unit, IARI were used at 1:1000 dilutions, and anti-rabbit IgG alkaline phosphatase conjugate (Sigma, USA) was used at 1:20,000 dilutions. The reaction of ELISA was read at 405 nm after 30, 60 and 90 min of addition of substrate, *p*-Nitrophenyl Phosphate (PNPP, Sigma, USA) by using ELISA reader, ELX 800 MJ (BioTek Instruments Inc, USA).

Results and Discussion

Symptomological study

Symptoms studied under field conditions revealed that the disorder mainly affects flower development and leads to either no or deformed buds (Fig. 1a). From this earlier report, TSV reduced the number of pods per plant and delayed seed maturation. Other symptoms include stunting, bud blight, leaf mosaic (mottling), dwarfed leaves and stem discoloration (Mueller and Erika, 2013)**.** Field view of infected soybean plant showed thicker and dark green stem (Fig. 1b). Another virus-like conspicuous symptom was the chlorosis and necrosis of deformed leaves, and a few plants showed thickening, twisting and swollen stems (Fig. 1c). Earlier reported the same kind of shortened and thicker stem (Reddy *et al.*, 1995). We had also observed the plant height of symptomatic plant is shorter than the asymptomatic plants (Table 1 and Fig. 2). This result showed positive with the findings of Jain *et al.*, (2008), where they reported necrosis of leaf lamina, petiole, stem and floral bud.

These symptoms are also similar to the findings of Jadhav *et al.*, (2013) who collected sample from different regions of Maharashtra.

Table.1 Comparison of symptomatic and asymptomatic soybean plants based on size of stems, internodes and plant height

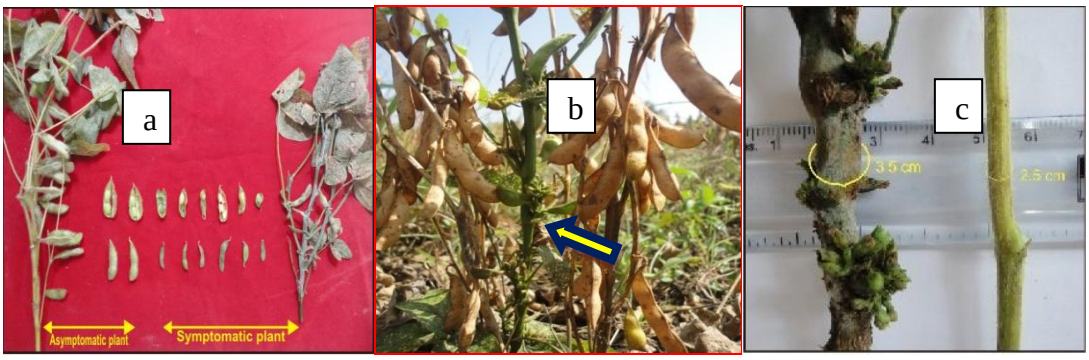
Sl. No.	Samples	Thickness of stem (cm)	Length of internodes (cm)	Plant height (cm)
1	Asymptomatic	2.4 cm	6 cm	45 cm
	Symptomatic	3.5 cm	2 cm	30 cm
2	Asymptomatic	2.5 cm	6 cm	40 cm
	Symptomatic	3.2 cm	2.5 cm	25 cm
3	Asymptomatic	2.4 cm	5.5 cm	45 cm
	Symptomatic	3 cm	2.4 cm	25 cm
4	Asymptomatic	2.4 cm	6 cm	35 cm
	Symptomatic	3.1 cm	3 cm	15 cm
5	Asymptomatic	2.5 cm	7 cm	50 cm
	Symptomatic	3.7 cm	2.5 cm	39 cm

Table.2 DAC-ELISA diagnosis of symptomatic FBD of soybean samples from different regions of Vidarbha (Maharashtra)

Samples	Location	Genotype	GBNV	TSV
1	MS-AK-PDKV-2	JS-335	-	+++
2	MS-PDKV-3	JS-335	-	++
3	MS-PDKV-4	JS-335	+++	+++
4	MS-AMT-RRC, Amravati	JS-335	++	+++
5	MS-Buldhana	JS-335	+	+
6	MS-BUL-Nandura	JS-335	+	++
7	MS-AK-Wanirambapur-5	JS-335	-	+
8	MS-AK-Wanirambhapur-6	JS-335	-	++

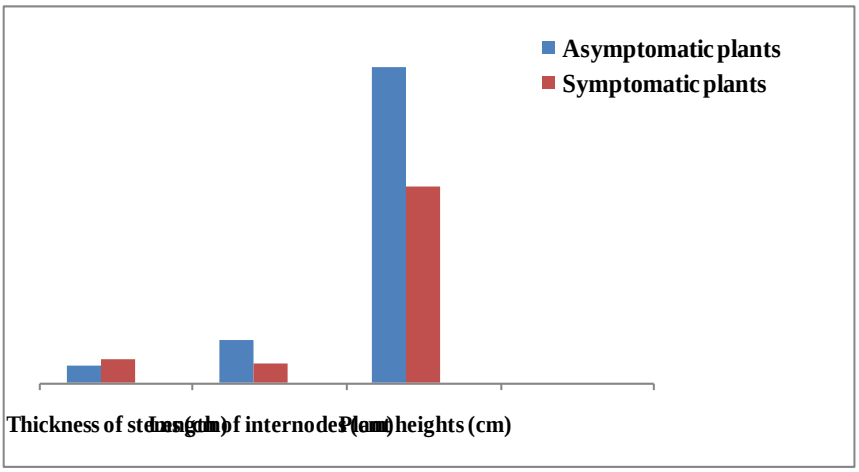
Note: +: Positive reaction, -: Negative reaction, ++: Strong reaction, +++: Very strong reaction

Fig.1 Symptoms of floral bud distortion in symptomatic soybean plant



a: Reduction in pod size of symptomatic soybean plants; b: Field view of symptomatic soybean plant with deformed pods; c: Thicken stem of symptomatic plant

Fig.2 Columns showing the mean comparison of symptomatic and asymptomatic plants based on size of stems, internodes and plant heights



The symptomatic plants produced thicker stem and shorter internodes as compared to the asymptomatic plants. GBNV infected plants exhibited chlorotic spots surrounded by irregular brown necrotic margins on leaves, and necrosis of petioles and stems reported by Akram *et al.*, (2013). Earlier reported symptoms of TSV include chlorosis and necrosis of leaves, necrotic streaks on petioles, stems, floral parts and stunted growth (Kumar *et al.*, 2008).

DAC- ELISA

DAC-ELISA was employed by using the antiserum raised against TSV and GBNV to identify the actual causal virus of floral bud distortion of soybean. The samples collected from different locations of Vidarbha region, Maharashtra, were sent to IARI, New Delhi, for detection of the association of TSV and GBNV in infected soybean samples. All eight samples showed positive reaction. Positive reaction was observed in both positive controls i.e., TSV infected soybean with TSV antiserum and GBNV infected groundnut with GBNV antiserum. Both negative controls i.e., buffer and healthy samples exhibited negative reactions. The test revealed a strong positive reaction with TSV antiserum and GBNV antiserum indicating TSV and GBNV as the causal viruses of floral bud distortion of soybean (Table 2). Similarly, Fagbenle and Ford (1970) reported infection of soybean with TSV as there was positive test with antiserum of TSV. Virus was partially purified and confirmed by DAC-ELISA which confirmed the causal virus as TSV (Baswaraj, 2010). Kumar *et al.*, (2008) also reported the association of TSV with infected soybean plants with chlorosis and necrotic lesions on leaves, veins, mid-rib, petioles, stem and pods reacted positive with the TSV polyclonal antiserum in DAC-ELISA. GBNV infection in urdbean has been reported earlier from various parts of India using DAC-ELISA by Bhat *et al.*, (2001) and Biswas *et al.*, (2009).

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