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Early detection of soybean crinkle problem: Biotic or abiotic factor?

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Abstract
Detection of Begomovirus infecting soybean was carried out during *Kharif* 2018 using specific begomovirus primers. The leaves showing crinkle symptoms was used for the early detection of the virus. There was no amplication of DNA in tested samples which clearly indicated the absence of virus. Molecular dectection can be successfully used for early diagnosis of the cause and helps in reducing in cost of cultivation.

Keywords: Early detection, soybean crinkle, Biotic, abiotic factor

Introduction
Soybean (*Glycine max* (L.) Merril) is world's most important seed legume, which contributes about 25% to the global edible oil production, about two thirds of the world's protein concentrate for livestock feeding. Presently in India it is cultivated over an area of 10.56 m ha with an annual production 12.22 mt (Anon., 2017) [1]. In Karnataka also the area under soybean cultivation is increasing and has reached 0.27 m ha. with a production of 0.17 mt. Many biotic and abiotic problems have been encountered in soybean production. During *Kharif* 2018, in Northern Karnataka region, soybean cultivation was maximum, but in some varieties showed crinkle symptoms in farmer's field and experimental plots. In recent years, soybean yellow mosaic virus disease is becoming problematic in North and Central India. The symptoms of Soybean Yellow Mosaic diseases (SYMD) depend on the host genotype, virus strain, plant age at the moment of infection and the environment. Field symptoms can range from apparently asymptomatic plants to severely mottled and deformed leaves. Leaves can appear curly or waved and some cultivars show necrotic local lesions that can later merge into veinal necrosis followed by yellowing and leaf abscission. Some strains can cause severe stunting, systemic necrosis, leaf yellowing, petiole and stem necrosis, terminal necrosis and crinkle (Hill, 2014) [3].

In Central India, Yellow Mosaic Disease (YMD) of soybean is caused by Mungbean Yellow Mosaic India Virus (MYMIV) (Usharani *et al*., 2004) [4] which belong to genus *Begomovirus* of family *Geminiviridae* and transmitted by insect vectors whiteflies and causing severe yellow mosaic disease (YMD) in tropical and subtropical conditions. In this context, to ascertain whether crinkle symptoms is due virus or any other environmental conditions, the molecular detection was done for the presence of Begomovirus which is quick and reliable answer to take up precautionary management practices.

Material and methods
Leaves of soybean showing typical cirnkle symptoms along with the healthy leaves were collected from research plots at University of Agricultural Sciences, Dharwad, Karnataka, and also farmers field of the Bailhongal taluk (Belagaum District). DNA extraction protocol suitable for small amount of plant tissues was followed to isolate total DNA from crinkle and healthy leaf samples (as negative control) (Doyle and Doyle, 1987) [2]. Total DNA extracted was used as a template in polymerase chain reaction (PCR) to detect the occurrence of begomovirus. Oligonucleotide primers used for detecting begomovirus are (BegomoF 5'ACGCGTGCCGTGCTGCTGCCCCATTGTCC3' and BegomoR 5' ACGCGTATGGGCTGTCGAAGTTGAGAC 3').

The reaction was carried out in 20 µl volumes, which contains 1.0µl (25ng) of soybean genomic DNA, 1.0µl (2.5pmole) of forward and reverse primers each, 1.0µl (2.0mM) of dNTPs, 2.0µl of Taq buffer (10X), 1.0µl of MgCl2 (25mM) and 1 units of Taq polymerase. All the chemicals and plastic wares used were obtained from Genei and Tarsons respectively. The thermal cycler conditions for virus gene amplification involve 1 cycle of DNA

denaturation at 94 °C for 5 min followed by 35cycles each having a denaturation at 94 °C for 60 second, followed by annealing (68-71 °C for 60 seconds depending on the PCR amplicons) and a primer extension at 72 °C for 60 second and by final extension of 72 °C for 10 min. The PCR products was resolved in 1.0% agarose gel electrophoresis in 1X TAE buffer and an aliquot of 100ng of 100 bp DNA ladder (Himedia) used as molecular size marker and visualized by ethidium bromide staining.

Results and discussion

In this study, early detection and diagnosis of begomovirus species infecting soybean was carried out in order to employ suitable management practices in the farmers field (Fig. 1). Polymerase chain reaction performed using Begomovirus coat protein-specific primers to check the presence of yellow mosaic virus which belong to Begomovirus group. There was no amplification of 200 bp amplicon in case of sample no 3 (Soybean sample showing crinkle symptoms) and sample no 4 (Soybean healthy sample) when compared to the sample no 1&2 (Begomo virus positive control) clearly indicated that the test sample is free from begomo virus infection. (Fig. 2)
Presence and detection of Begomovirus in soybean ecosystem is concern for soybean growers. So, early detection was done by PCR assay to ascertain the presence of Begomovirus but the results clearly indicated the absence of virus in the crinkled leaf samples. Hence no management practices were advocated, later during the flowering stage of the crop there was disappearance of the crinkle symptoms and there was no significant difference in the yield data which may be attributed to the some abiotic factors. Hence, such early detection helps to reduce the cost of cultivation for the soybean growing farmers.

Leaves showing crinkle symptoms Healthy leaves

Fig 1: Soybean leaves showing crinkle symptoms

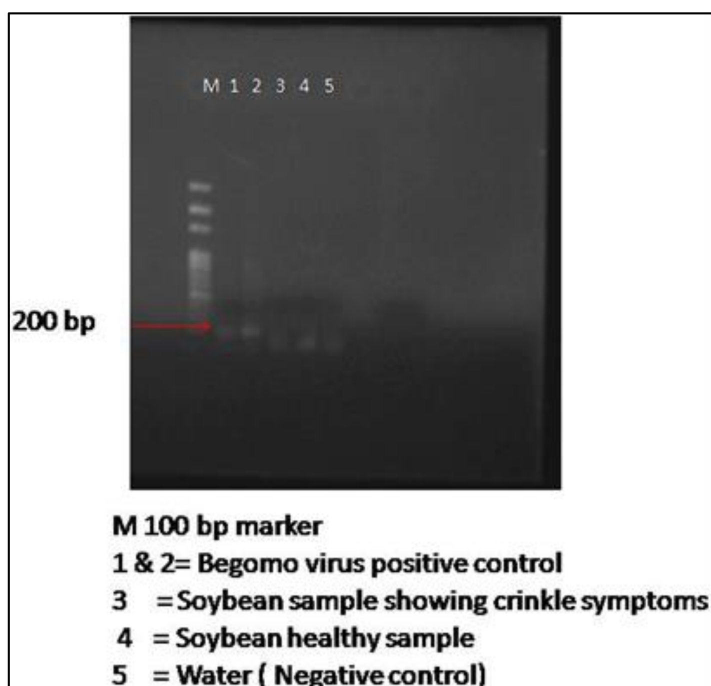

Fig 2: Molecular detection for the presence of Begomovirus in crinkled leaves of Soybean

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