Detection of phytoplasmas causing grassy shoot disease in sugarcane by immunofluorescence technique

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Grassy shoot disease of sugarcane caused by phytoplasmas was first observed in India by Barber (3) and reported by Vasudeva (9) from Belapur. Later, the disease was reported from most of the sugarcane growing tracts in India (1). This disease causes severe loss as number of millable canes in plant crop and its impact is much severe in ratoon crop. The disease intensity may vary among the varieties but up to 100% incidence in the susceptible variety Co 8021 was recorded recently in Tamil Nadu (Viswanathan, Unpublished). To prevent severe losses under field conditions, selection of disease free seed material is of paramount importance. Presently disease free canes were selected, based on the characteristic disease symptoms observed under field conditions. However, this is time consuming and expensive also. Sometimes the typical disease symptoms may not be produced due to varietal or environmental factors. So, improved techniques are needed to be developed to index the seed canes to prevent future loss both in plant and in ratoon crops. Recently some improved serological techniques with more precision were standardized for detecting GSD phytoplasmas in infected sugarcane tissues (11, 13). This paper reports the indirect immunofluorescence technique developed for the detection of GSD phytoplasmas in sugarcane.

Infected sugarcane material was collected from the Institute Farm and maintained at the Plant Pathology glass house in pots. For purification of phytoplasmas, young leaves showing albino symptoms were selected. The phytoplasmas were purified from the infected leaves following the method of Clark et al. (5) with certain modifications as previously reported by Viswanathan and Alexander (13). Infected leaf materials were initially ground in GM buffer (0.3 M/1 glycine-NaOH, pH 8.0 and 0.02 M/1 MgCl$_2$). The homogenate was centrifuged at 8000 rpm and the supernatant was centrifuged at 60,000 rpm for 60 min. (Sorvall). The pellets were dissolved in 1 ml of GM buffer and after clarification, the preparation was incubated with 10% (v/v) rabbit antiplant immunoglobulins (IgG) for 18 h at 4°C to reduce contamination by plant antigens. After clarification at 2000 rpm for 5 min. rabbits were injected intramuscularly at 10 to 12 day intervals over a period of 8 to 10 weeks with a 1:1 mixture of the partially purified phytoplasma preparation and Freund’s adjuvant (incomplete). Animals were bled 10 days after the last injection from the marginal vein and booster injection and further breeding were made as required.

Indirect immunofluorescence technique was done following the method of Seemuller (8). Very fine hand sections were cut from infected and healthy sugarcane tissues for the fluorescent antibody technique (FAT). Initially, the sections were immersed in phosphate buffered saline (PBS) (pH 7.2, 0.1 M) for 30 min. Later, tissue sections were immersed in the phytoplasma antisera diluted to 1:100 in PBS for 60 min. at room temperature. After incubation the tissue sections were washed with PBS three times at 3 min. intervals. Later, the tissue sections were immersed in antirabbit gamma globulins conjugated with fluorescein isothiocyanate (FITC) (Sigma, USA) diluted to 1:100 in PBS for 60 min. under darkness. After incubation the tissue sections were washed as previously and fixed in 10% glycerol in PBS and the tissue sections were viewed under fluorescent microscope (Zenalumer) using UV filter BP 450-490 mm, IP 520 nm. For each treatment at least 5 sections were observed.

The results of the present studies clearly established that the FAT could be a useful technique in the detection and localization of phytoplasmas causing GSD in infected sugarcane tissues. Usually phytoplasmas...
colonize the sieve tubes of vascular strands (4). In sugarcane also same was observed under the fluorescent microscope. Usually the infected tissues exhibited apple green fluorescence confined to vascular bundles and healthy tissues showed no such fluorescence. Apple green fluorescence in the infected tissue bundles is due to the specific bindings of FITC - conjugate with the phytoplasmas colonizing the sieve tubes. Initially internodal tissues from young tillers were utilized for the standardization. In the study 1 : 100 dilution of phytoplasma antiserum and FITC-conjugate was found optimum for the reliable detection of the pathogen. In subsequent studies, tissue samples from different parts of the sugarcane plant (variety Co 8021) such as leaf lamina, leaf midrib, leaf sheath, nodes and root were screened for phytoplasmal colonization. All the tissue samples clearly exhibited the fluorescence, confirming the phytoplasmal infection in all the plant parts (Fig. 1).

The systemic colonization of all parts by phytoplasmas indicates the systemic nature of the disease in the host. Previously, Velmurugan (10) found colonization of phytoplasmas in the infected plant leaves, stem and roots by electron microscopic studies. The present study also confirms the systemic nature of the pathogen. Since, the pathogen systemically colonizes the host, indexing for this pathogen is a must in selecting cane samples for healthy seed programme. If the cane is intended for quarantine, then sensitive technique like FAT has to be employed for reliable detection. Sugarcane Breeding Institute also maintains world sugarcane germplasm collections, where quarantine for sugarcane pathogens is routinely done. Earlier studies (11, 12) indicated that two ELISA systems can be applied for the detection of the GSD pathogen in sugarcane. The ELISA gives the titre values of the phytoplasmas in the tissue, whereas immunofluorescence shows the exact colonization of the sieve tube elements by the pathogen. Since the localization of the phytoplasmas in particular tissues is possible in FAT, this has certain advantages over the ELISA technique.

As most of the sugarcane diseases are carried through seed cane, using infected seed materials may lead to significant loss in sugarcane yield. Further, the loss due to diseases is much more in ratoon crops. In general, severity of GSD will be more in ratoon crops than in plant crop (7,10). Although use of fluorescence antibody technique under field condition seems to be difficult, it can be used at the research stations and sugar factories, where the primary/breeder seed is produced and supplied to the farmers. If the primary seed material is free from GSD and other diseases, the crop can be maintained free from diseases employing three-tier seed nursery programme (2). Time to come, this fluorescent antibody technique will have greater applications in the healthy seed programme. Detection of phytoplasmal diseases by FAT in other crops has also been reported (6,8,14). The FAT was also applied in the detection of phytoplasma colonization in insect vectors and insect transmission of the phytoplasmas (6). In sugarcane also, this technique can be employed to study the secondary transmission of the disease by different insect vectors under field conditions. In addition to ELISA, FAT will have more applications in the field of GSD detection and indexing sugarcane clones for healthy seed programme and germplasm exchange.

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REFERENCES


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