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Detection of Sugarcane Yellow Leaf Virus in the Infected Sugarcane Plant by DAC-ELISA

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ABSTRACT

Sugarcane Yellow Leaf Disease (SCYLD) is a viral disease caused by *Sugarcane Yellow Leaf Virus* (SCYLV) affecting sugarcane and posing a serious threat to the sugarcane cultivation. Management of yellow leaf disease is possible through planting disease-free planting-materials indexed through sensitive diagnostic methods and the development of diagnostic methods helps in detection of SCYLV in asymptomatic plants and suspected plant material. In the present study, polyclonal antibodies against recombinant coat protein were produced for SCYLV and used for detection of the virus in different parts of the infected plant. Using the antibodies, a study regarding the distribution of SCYLV in various tissues and leaves of infected sugarcane plant and plants raised from meristem tip culture were analyzed by DAC-ELISA. The antibodies raised against recombinant coat protein of SCYLV were used at 1:5,000 dilution. Virus distribution in four different tissues of the susceptible variety *i.e.,* 2003V46 (infected and crop developed from meristem tip culture) and a tolerant variety for SCYLV i.e., 97R167 was studied using DAC-ELISA. The samples were taken from 6-8 months old crop. Out of 12 different tissues/ leaf positions tested for SCYLV presence, maximum OD405 values were observed with +1 mid rib leaf samples of infected var. 2003V46 (1.178) followed by the stem sap (0.893), +4 mid rib leaf samples (0.391) and the least in roots (0.083). The SCYLV tolerant var. 97R167 also showed least OD405 values ranging from 0.068-0.093. Virus was also detected in asymptomatic plants raised by meristem tip culture and whose A405 values are twice the negative control. The results from the study clearly demonstrated that higher concentration of SCYLV in midribs of +1 infected leaves as compared to other tissues of plant and hence midrib of +1 suspected leaf may be taken for diagnostic purpose.

Keywords: *Distribution, Sugarcane, DAC-ELISA, Diagnosis and Sugarcane yellow leaf virus.*

Yellow leaf disease (YLD) of sugarcane caused by *Sugarcane Yellow Leaf Virus* (SCYLV) is posing a serious threat to sugarcane cultivation affecting production and productivity of many ruling sugarcane varieties in India causing losses ranging from 60 (first crop) to 100% (ratoon crop). The disease is reported worldwide in more than 30 countries (Lockhart and Cronje, 2000 and Schenck, 2001). In India,Viswanathan *et al.* (1999) reported the disease for the first time and at present, the disease has spread and prevalent in major sugarcane growing states like Andhra Pradesh, Karnataka, Tamilnadu and Madhya Pradesh (Viswanthan, 2002; Hemalatha *et al.*, 2014; Viswanathan and Rao, 2011; Suresh *et al.*, 2016)

In Andhra Pradesh sugarcane is grown in an area of 1.26 lakh hectares with average production of 76.14 t ha^{-1} (Source: Cooperative Sugar, Feb,

2020). At present the disease is spreading at an alarming stage infecting almost all the varieties grown by the farmers in Andhra Pradesh. The disease has spread to number of ruling varieties like, 2003V46, Co 86032, 83V15, 87A298, 86V96, Co 62175, 2002V48, 2005T16. In Andhra Pradesh, yellow leaf disease symptoms were observed since 2004 in the farmers' fields of Nizambad (Bharathi and Kishanreddy, 2007) and later observed in the co-ordinated trials at Regional Agricultural Research Station, Anakapalle. In Chittoor district, the disease was first observed in the experimental plots of Agricultural Research Station Perumallapalle, during 2009-10 in the entries Co 7219 and 87A298 and a number of varieties got affected in the AICRP trials. During 2010-11, the disease soon appeared in the farmer fields in almost all the sugarcane growing areas of the District. The popularly grown sugarcane varieties the state (2003V46, 86V 96, CO 6907, Co 86032, 87A298, 83V15) were infected with the disease.

Continuous cultivation of a single variety over a large area and multiple ratooning are the major factors responsible for spread of systemic diseases like red rot, smut, YLD and mosaic. The highest incidence of the yellow leaf disease was recorded in the ratoon fields of East Godavari district (51.0%) with a mean incidence of 40.8% followed by Chittoor (40.0% and 35.0%) and the least mean incidence was observed in Visakhapatnam (17.0%) than in Vizianagaram district (26.3%) (Suresh *et al.*, 2020).

SCYLV is a Polerovirus with single stranded RNA genome limited to phloem. Development of precise diagnostic techniques to detect the virus in asymptomatic stage is warranted under Indian conditions since seed canes are cut in the age of six to seven months. Symptom expression during such stage is not expected under field conditions hence this technique will be useful to identify the virus free seed canes. Supply of disease free seed materials ensures freedom from YLD and it would ultimately sustain sugarcane productivity under Indian conditions. In this regard, elimination of the virus through meristem culture combined with molecular diagnosis was demonstrated. Currently RT-PCR technique was used to diagnose SCYLV in tissue culture-derived plantlets and in germplasm varieties. However, RT-PCR technique is not suitable for indexing large numbers of samples due to the costs and relative complexity of execution. Therefore, serological tests particularly Enzyme-Linked Immunesorbent Assay (ELISA) has been commonly used for screening a large number of samples. SCYLV can be detected by both serological (Scagliusi and Lockhart, 2000) and molecular methods (Comstock *et al*., 1998). Although molecular methods are more sensitive than serological ones, their use on a large scale for routine diagnosis is more expensive, the use of polyclonal antisera has contributed greatly to the detection of the Luteovirus by direct ELISA and TBIA (Schenck, 1997; Comstock *et al.*, 1998; Moutia and Saumtally, 2001). SCYLV often persists in plants without being noticed by the growers and this asymptomatic stage seems to be the most common epidemiological state for the virus spread through vegetative cuttings. Hence, for detection of the virus in plants showing asymptomatic symptoms, the choice of plant part for disease diagnosis plays an important role and in the current study deals with the tissue to be used for diagnosis of the virus by DAC-ELISA.

MATERIAL AND METHODS

Plant samples used for detection

Sugarcane leaf samples from eight months old plants of three different sugarcane material maintained at Agricultural Research Station, Perumallapalle ie., infected leaves of 2003V46, meristem tip culture raised from the 3rd ratoon of the variety 2003V46

and YLD tolerant genotype 97R167 were used for analyzing the virus distribution with in the sugarcane plant during 2020-21. From each sample, four different plant tissues ie., +1 midrib, +4 midrib, stem sap and roots were analyzed for the virus titre by DAC-ELISA.

Preparation of samples

Two types of leaf samples {+1 midrib (top most leaf) +4 midrib (4^{th} leaf from the top)} of the suspected sugarcane were used. Leaf samples were powdered by grinding in a mortar and pestle with liquid nitrogen in extraction buffer (100 mM phosphate buffer, pH 6.0) containing 1% Na_2SO_3 and 0.05% Tween 20 (1:50 dilution), centrifuged and leaf extracts were obtained. Using a sugarcane piercer, 2.0 ml of stem sap was collected from the third internode from the top in microfuge tubes and was placed on ice. The samples were centrifuged briefly to remove the solid particles and preserved at -20!. Samples from infected susceptible variety 2003V46 was used as positive check while samples from healthy cane of meristem tip raised crop (Plant crop) was used as negative check in the assays.

Detection of SCYLV by DAC-ELISA

The protocol suggested by Hobbs *et al.* (1987) was followed for the detection of SCYLV in sugarcane leaves by DAC-ELISA. The samples (infected leaves and roots) were grinded in carbonate buffer at the rate of 100 mg ml^{-1} and 100 µl was dispensed into each well of the ELISA plate. The stem sap was directly added to the plate as antigen. The plates were incubated the at 37°C for an hour or kept at 4°C overnight. The plate was washed with three changes of PBS-T, allowing three minutes per each wash. Antiserum of purified polyclonal antibodies of SCYLV produced in the Department of Plant Pathology, IFT, RARS, Tirupati using recombinant approach was used in the study at a dilution of 1:5000 in PBS-TPO buffer and dispensed 100 µl into each well of ELISA plate and the plate was incubated at 37°C for an hour or left at 4°C for overnight and the plate was washed with three changes of PBS-T. Secondary antibody ie., diluted anti-rabbit-ALP-Conjugate @ 1:15000 (Sigma) in PBS- TPO was dispensed @ 100 µl into each well of ELISA plate and incubated the plate at 37°C for an hour or left at 4°C for overnight. The plate was washed with three changes of PBS-T and was added with 100 µl of PNPP substrate into each well. The plates were incubated in dark at room temperature. The absorbance was noted at 405 nm in an ELISA plate reader (BIORAD). The positive reaction was recorded when colourless substrate turned into light yellow later to dark yellow colour. The reaction was stopped by addition of 50 µl 3M NaOH per well. The readings were considered positive if the values were at least twice those of controls.

RESULTS AND DISCUSSION

The samples were taken from 6-8 months old crop. Out of 12 different tissues/ leaf positions tested for SCYLV presence, maximum OD405 values were observed with +1 mid rib leaf samples of infected var. 2003V46 (1.178) followed by the stem sap (0.893), +4 mid rib leaf samples (0.391) and the roots (0.083) (Table.1). The other two genotypes which didn't show any symptoms of the disease visually but showed positive to the virus in DAC-ELISA and recorded lower absorbance values ranging between 0.068- 0.136. However the virus titre was high with respect to the +1 leaf midribs among all the three types of genotypes and the lowest was in the roots. From the results it was evident that even the healthy crop raised from meristem tip culture showed the presence of virus at lower concentrations at later generations. SCYLV often persists in the plants without being

Table 1. Detection of SCYLV in different tissues of three types of cultivars

S. No.	Cultivar and type of tissue taken for detection	Visual symptom	OD values at 405nm
1	2003V46-Infected +1 midrib	+	1.178
2	2003V46-Infected +4 midrib	+	0.391
3	2003V46-Infected-stem sap	+	0.893
4	2003V46-Infected- roots	+	0.083
5	2003V46-Meristemtip raised plants+1midrib	-	0.099
6	2003V46-Meristemtip raised plants + 4midrib	-	0.076
7	2003V46-Meristemtip raised plants -Stem sap	-	0.136
8	2003V46-Meristemtip raised plants-roots	-	0.087
9	97R167- +1 midrib	-	0.093
10	97R167- +4 midrib	-	0.091
11	97R167-stem sap	-	0.088
12	97R167-roots	-	0.068
13	Positive control (infected)		1.98
14	Negative control (healthy)		0.045

noticed by the growers. In fact, this non-symptomatic stage seems to be the most common epidemiological state for this viral pathogen. Usually YLD symptoms are expressed in 6–8 months in the field (Viswanathan *et al.*, 2009). Screening of SCYLV in Hawaii revealed positive infection in all susceptible sugarcane cultivars, but disease symptoms appeared only occasionally (Schenck and Lehrer, 2000). However, this nonsymptomatic stage can still lead to significant yield declines (20–30%) (Rassaby *et al*., 2003). Scagliusi *et al.* (2009) revealed that the midrib regions of younger leaves is best tissue for detection of SCYLV infection. He also reported higher virus concentration in the midribs of younger leaves (+1 leaves) than leaf blades and significantly lower concentration of SCYLV in both midrib and leaf blade of +4 leaves. Moutia and Saumatlly (2001) standardized technique for diagnosis of SCYLV from infected canes by ELISA and it was established in the study that SCYLV is present in all the infected stalks. It was also reported that the virus concentrations in mature stalks (10 – 12 months old), tends to be concentrated in the upper third of the stalk and is more easily detected there. Alternatively, juice samples were also reported to be best sample for analyzing the virus titre. Schenck *et al.* (1997) reported that juice samples from upper portion of the stalk are positive in many cases than those from bottom portion. Since the virus is phloem – limited, the method of using juice as a starting material will be a more convenient system.

LITERATURE CITED

Bharathi V and Kishan Reddy L 2007 Mixed infection of *Sugarcane streak mosaic* and *Sugarcane yellow leaf virus* infecting sugarcane crops in Andhra Pradesh, India. *International Journal of Agricultural Sciences,* 3(1): 68-72.

Comstock J C, Irey M S, Lockhart B E L and Wang Z K 1998 Incidence of yellow leaf syndrome in CP cultivars based on polymerase chain reaction and serological techniques. *Sugarcane*, 4: 21-24.

Hemalatha T M, Hemanth Kumar M, Afsar S, Bhaskar Reddy B V and Subba Rao M 2014 Molecular detection of Sugarcane Yellow Leaf Virus in popularly grown sugarcane varieties in Andhra Pradesh and characterization of the virus from the Sugarcane

variety 2003V46 *Journal of Mycology and Plant Pathology,* 44(4): 442-446.

Hobbs H A, Reddy D V R, Rajeswari R and Reddy A S 1987 Use of direct antigen coating method and protein-A coating ELISA procedures for detection of three peanut viruses. *Plant Disease,* 71: 747- 74.

Lockhart B E L and Cronje C P R 2000 Yellow leaf syndrome. In: Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally AS (eds). *A guide to sugarcane diseases*. CIRAD-ISSCT, Montpellier, 291-295.

Moutia J F and Saumtally S 2001 Diagnosis of sugarcane yellow leaf virus in cane juice and the effect of hot water treatment on its control. *Proceedings of International Society of Sugar Cane Technologists,* 24: 444-450.

Rassaby L, Girard J C, Letourmy P, Chaume J, Irey M S and Lockhart B E 2003 Impact of sugarcane yellow leaf virus on sugarcane yield and juice quality in Reunion Island. *European Journal of Plant Pathology,*109: 459–466.

Scagliusi S M M and Lockhart B E L 2000 Transmission, characterization and serology of a Luteovirus associated with yellow leaf syndrome of sugarcane. *Phytopathology,* 90: 120-124.

Scagliusi S M, Basu S K, Gouvea J A and Vega J 2009 Comparison of two diagnostic methods for evaluation of *Sugarcane yellow leaf virus* concentration in Brazilian Sugarcane cultivars. *Functional Plant Science and Biotechnology,* 26-30.

Schenck S 1997 Advances in control of yellow leaf syndrome. Hawaii Agriculture Research Center. *Pathology Report,* 67: 1-4.

Shenck S and Lehrer A T 2000 Factors affecting the transmission and spread of sugarcane yellow leaf virus. *Plant Disease,* 84: 1085-1088.

Suresh M, Raja Kumar N, Adilakshmi D, Kishore Varma, Bharatalakshmi P and Veerabadhra Rao K 2016 Detection of sugarcane yellow leaf virus (SCYLV) in field varieties and in *in vitro* regenerated plantlets of sugarcane through DAS-ELISA. *Sugar Journal* 46th *Annual convention of SISSTA*, 44-49.

Suresh M, Umadevi G, Raja Kumar N and Tirupathi M 2020 Current Status of Yellow Leaf Disease (YLD) of Sugarcane Caused by Sugarcane Yellow Leaf Virus (SCYLV) in the states of Andhra Pradesh and Telangana. *International Journal of Bio-resource and Stress Management,* 11(1): 073-081.

Viswanathan R, Karuppaiah R, Malathi P, Ganesh Kumar V and Chinnaraja C 2009 Diagnosis of *Sugarcane yellow leaf virus* in asymptomatic sugarcane by RT-PCR. *Sugar Tech,* 11: 368-372.

Viswanathan R, Padmanaba P, Mohanraj D, Ramesh Sundar A, Premachandran M N 1999 Suspected yellow leaf syndrome in sugarcane. *Sugarcane Breeding Institute Newsletter,* 18(3): 2-3.

Viswanathan R 2002 Sugarcane yellow leaf syndrome in India: incidence and effect on yield parameters. *Sugarcane International,* 20(5): 17-23.

Viswanathan R and Rao G P 2011 Disease scenario and management of major sugarcane diseases in India. *Sugar Tech,* 13: 336–353.

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