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Management of yellow leaf disease of sugarcane through meristem culture

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Abstract
Sugarcane yellow leaf disease (YLD) is one of the important viral disease in sugarcane. It is caused by Sugarcane yellow leaf virus (ScYLV), a positive sense single stranded RNA virus primarily transmits through infected setts and secondarily transmits through aphid *Melanaphis sacchari.* Earlier, it was identified as a minor disease in India, but in recent years it attained the epidemic status with the disease incidences from 30 to 50% in plant crop and more than 70% in ratoon crops which significantly affected the crop productivity. The most characteristic symptom of YLD is a distinct yellowing of the lower surface of the leaf midrib on young leaves at the apex of the mature plants, which can extend laterally to the leaf lamina. Conventional disease management approaches including chemical treatments and cultural practices have proven inadequate due to systemic and persistent nature of causal agents, often phytoplasmas or viruses. Meristem culture, a tissue culture based technique, offers a promising alternative for the production of disease free planting material by exploiting the fact that meristematic tissues are often free of systemic pathogens, phytoplasmas or viruses. Tissue culture plantlets of two varieties *viz.,* Co 86032 and VSI 08005 were transplanted and MHAT (Moist Hot Air Treatment at 54 ^{0}C) treated two varieties *viz.,* Co 86032 and VSI 08005 and control of two varieties Co 86032 and VSI 08005 were planted at VSI farm and were observed throughout the year for the natural incidence of YLD. The incidence of YLD was observed in all treatments during the year except tissue culture plantlets of Co 86032. However, the incidence of YLD noticed late in plot where TC plantlets ad MHAT treated seedlings are used. This seems that there may be secondary spread of the disease due to some sucking pests. The resulting plantlets were evaluated through molecular diagnostics and symptom observation confirming the absence of YLD. This approach demonstrates the potential of meristem culture as a sustainable and efficient method for managing YLD.

Keywords: Aphid, Sugarcane, yellow leaf disease, yellow leaf virus, yellowing

Introduction
Sugarcane (*Saccharum* spp.) is one of the most important commercial crops grown mainly for sugar in many countries and also for bio-energy production from its by-products such as, bagasse and molasses. Sugarcane is a very useful asset for economic developments in different tropical and subtropical areas of the globe including India. Its production faces several biotic stresses, among which viral diseases are of critical concern. Yellow Leaf Disease (YLD) is caused by Sugarcane yellow leaf virus (SCYLV), a member of the genus Polerovirus (family Solemoviridae). Yellow leaf disease (YLD) of sugarcane was first reported in Hamakua (Hawaii) on variety H65-0782 in 1989 as yellow leaf syndrome (Schenck, 1990 and Schenck *et al.*, 1997) [6, 8] and subsequently from the United States mainland (Comstock *et al.* 1994) [2] and many other sugarcane growing countries. YLD is reported worldwide in more than 30 countries (Lockhart and Cronje, 2000, Tran-Nguyen *et al.*, 2000 and Schenck, 2001) [3, 10, 7]. In India, Viswanathan *et al.* (1999) [14] reported the disease for the first time and the associated Sugarcane yellow leaf virus which assumed its severity on different sugarcane varieties. In India, the disease is prevalent in major sugarcane growing states like Andhra Pradesh, Karnataka, Tamilnadu and Madhya Pradesh (Viswanthan, 2002; Suresh *et al.*, 2014; Viswanathan and Rao, 2011) [12, 9, 13]. Continuous cultivation of a single variety and multiple ratooning are the major factors responsible for spread of systemic diseases like red rot, smut along with YLD and mosaic. Yellow Leaf Disease (YLD) posing serious problems during the recent past and severe losses reported in several sugarcane growing regions of both Andhra Pradesh and Teangana states.

Vega *et al.* (1997) [11] first identified the causative virus as *Sugarcane Yellow Leaf Virus* (SCYLV). The disease is widespread and difficult to control due to its systemic nature and vegetative propagation of the crop. Meristem culture has been widely adopted as a technique to eliminate systemic pathogens including viruses from plant propagation material.

Material and Methodology

I. Developing virus-free plants through meristem culture

i. Tissue culture

Methodology

Establishment of aseptic culture: Select the sugarcane variety for YLD-free seed production. Young cane tops are collected from 4-6 month old crop by removing the leaf sheath from field grown plants. The excised shoot tip of about 10 cm long is washed with water and then rinsed with a common disinfectant such as Savlon or Dettol solution followed by washing with sterilized water and dipping in 10% sodium hypochlorite solution for 10 minutes for disinfecting the plant material.

- **Inoculation of meristem tip:** A wide-mouth flask containing the surface sterilized material is taken inside the laminar flow chamber. The material is washed thoroughly 3-4 times with sterilized distilled water till the odour of chlorine fades away. The minimum possible size (about 2-5 mm) of apical dome is excised with help of a sterile sharp blade and placed in glass bottle containing modified MS medium supplemented with kinetin (0.015 mg/l) and benzyl adenine (1.0 mg/l) as well as sucrose (30 g/l). The apical domes (apical meristem) are incubated at 25° ± 1 °C under 16 hr / 8 hr light-dark cycle. The meristem is transferred to fresh medium once in 7-10 days for survival and growth. Initially, the growth would be slow and may take about 30 to 45 days for new shoots to come out.
- **Shoot multiplication:** The developing shoots are transferred to fresh containers with MS shoot multiplication medium for sub-culturing. A number of shoots emerge soon after and sub-culturing is repeated every 15 to 20 days depending upon the rate of shoot multiplication which may vary with the variety. After 45 to 60 days, the regenerated shoots are transferred to modified MS liquid medium along with kinetin (1.07 mg/ l) and benzyl adenine (0.25 mg/l) as well as sucrose (20 g/l). After 25-30 days, new shoots will arise from the axils of the developing shoots. The multiple shoots developed are separated in small groups and transferred to fresh multiplication medium once in 15-20 days. This process of subculture is repeated for 7-8 cycles until the desired number of shoots is attained.
- **Transfer of shoots to rooting medium:** Only well-grown shoots with three to four leaves should be transferred to rooting medium. Dry leaves are removed and green leaves trimmed at the tips. While separating, care is taken not to damage the basal portion of the shoots from where the roots would emerge. Groups of five to six shoots are placed in culture tubes containing half-strength MS medium supplemented with 5 mg/l naphthalene acetic acid and 30 g/l sucrose. Roots are formed within 15-25 days and once good root development has taken place the plantlets become ready for transfer to polybags/planting trays.
- **Hardening of plantlets:** Plantlets with well developed shoots and roots are taken out of the glass culture bottles and thoroughly washed with water to remove all traces of the medium. The plantlets with slightly trimmed roots and leaves are sown in polybags/planting trays containing a mixture of separately sieved river sand, silt and vermicompost or farm yard manure in a 1:1:1 ratio. The plantlets are maintained under intermittent mist or are covered with clean transparent plastic sheet until the first new leaves emerge. After 10 to 15 days under high humidity, the plantlets are transferred to shade net-house and maintained for another 4 to 5 weeks. NPK (1.0%) spray is given once in a week after establishment of the plantlets to improve initial growth. The plants become ready for transplanting in field after 45-50 days.

The canes produced in field from tissue culture-raised plants are designated as Breeder Seed which may be further multiplied for production of Foundation Seed and subsequently seed for commercial planting.

Indexing of plantlets for sugarcane yellow leaf virus (SCYLV)

Indexing of shoots before rooting may be carried out for SCYLV where facilities are available. The protocol is given below:

RT-PCR assays may be performed (Viswanathan *et al.* 2008, 2009). Total RNA is extracted from the first unfurled leaf along with midrib using TRI Reagent. The quality of RNA is checked in 1% agarose gel. The forward primer SCYLV-615F (ATGAATACGGGCGCTAACCGYYCAC) and the reverse primer SCYLV-615R (GTGTTGGGGRAGCGTCGCYTACC) may be used to specifically amplify ~613bp of the SCYLV genome. The total RNA to be reverse transcribed using RevertAid H Minus first strand cDNA synthesis kit (MBI Fermentas, USA), primed with 50 pmol of SCYLV-615R in a thermocycler. The PCR reaction to be performed in a total volume of 25 µl containing 2 µl cDNA, 2.5 µl of 10x PCR buffer containing 15mM $MgCl_2$, 0.5 µl of 10mM dNTP mix, 10 pmol each of forward and reverse primers (SCYLV-615F and SCYLV-615R, 1.25 units of *Taq*, and sterile milliQ water to the final volume.

PCR programme

Initial denaturation at 94°C for 4 min	
Denaturation at 94°C for 1 min	30 cycles
Annealing at 65°C for 1 min	
Primer extension 72°C for 45 sec	
Final extension72°C for 10 min.	

A 10 µl aliquot of each amplified product to be analyzed by electrophoresis on 1.5% agarose gel stained with ethidium bromide.

Impact of virus-free plants on crop growth and cane yield

Methodology

Plant randomized block design experiment with suitable replications involving virus-free plants of 2 to 3 varieties and their respective disease-affected canes or settlings in the

field. Make periodical observations on plant growth parameters and final yield parameters (including juice characters).

A study was conducted at VSI Research Farm, involving seedlings derived from tissue culture Co 86032, VSI 08005 (TC) and MHAT (Moist Hot Air Treatment at 54 ^{0}C) treated setts of Co 86032, VSI 08005 (MHAT) from Naigaon farm in MHAT device and control of Co 86032, VSI 08005 were planted at VSI farm in five rows of six meters length and were observed throughout the year for the natural incidence of YLD.

All the recommended package of practices was followed in raising a healthy crop. Matured canes of healthy and infected were harvested in each plot for recording yield components and juice quality parameters. Per cent brix, sucrose and purity were estimated at 24 hours intervals after harvest at 10th and 11th months of crop age following standard procedures (Meade and Chen, 1977) [7]. Yield components *viz*., total millable canes, total height of cane, millable height of cane (cm), number of internodes, girth of internode (cm), length of internode (cm), single cane weight (kg) at harvest were recorded on randomly selected canes while cane yield was recorded on plot basis. Reaction to YLD was carried out following 0-5 scale as per the methodology. Healthy and YLD infected leaf samples according to plot were collected and given to biotechnology laboratory for further detection of yellow leaf virus.

Data recording

Disease incidence was assessed using the scale developed by SBI, Coimbatore and AICRP on sugarcane (AICRP Annual Report 2014-15) as given in Table 1. The visual observation of the symptoms for assessing the disease spread both vertically and horizontally was assessed using the pictorial depiction of the disease grades as given in Figure 1. (AICRP Annual Report)

Table 1: Yellow Leaf Disease (YLD) severity grades

Disease grade	Description
0	No symptom of the disease
1	Mild yellowing of midrib in one or two leaves, no sign of typical bunching of leaves caused by YLD
2	Prominent yellowing of midrib on all the leaves in the crown. No bunching of leaves
3	Progress of midrib yellowing to laminar region in the whorl, yellowing on the upper leaf surface, and bunching of leaves
4	Drying of laminar region from leaf tip downwards along the midrib, typical bunching of leaves as a tuft
5	Stunted growth of the cane combined with drying of symptomatic leaves

(Source: AICRP on sugarcane-Annual Report 2014-15)

Fig 1: Symptoms of YLD displaying different severity grade (Adopted from AICRP annual report 2014-15)

Result and discussion

The experiment was conducted to evaluate the effectiveness of meristem culture in managing yellow leaf disease (YLD) in sugarcane. Observations on the presence of YLD were recorded for tissue culture (T.C.) derived plantlets, MHAT derived plantlets and untreated controls.

Data recorded on yield characters such as total millable canes, total height of cane, millable height of cane (cm), number of internodes, girth of internode (cm), length of internode (cm), single cane weight (kg) and juice quality parameters such as per cent brix, sucrose and purity were presented in Table 2, 3 and 4, respectively.

Table 2: Observations of Presence of Yellow Leaf Disease

Sr. No.	Genotype/Variety	Presence of YLD
1.	T.C. Co 86032	--
2.	T.C. VSI 08005	+
3.	MHAT Co 86032	+
4.	MHAT VSI 08005	+
5.	Control Co 86032	+
6.	Control VSI 08005	+

Table 2 showed that the meristem culture treatment was successful in eliminating YLD from Co86032 variety. Despite tissue culture, YLD symptoms were still observed in VSI 08005, indicating partial or no success of meristem culture in eliminating the pathogen for this genotype.

Heat treatment applied was ineffective for Co86032 and VSI 08005, with continued presence of YLD symptoms. Controls (Co86032 and VSI 08005) both showed YLD (+) as expected, confirming natural infection prevalence without any sanitation treatment. These results suggest that meristem culture alone may be an effective method for producing disease-free planting material but highlights the need for careful post-lab sanitation and monitoring to prevent reinfection and managing YLD in specific genotypes (like Co86032), but its efficacy varies across varieties. Additional or alternative sanitation techniques may be needed for genotypes such as VSI 08005. After testing of YLD infected and healthy leaf samples, absence of yellow leaf virus in micro propagated T.C. Co 86032 variety.

Table 3: Agronomic Yield Characteristics

Sr. No.	Treatment	Total Millable canes	Total Height (CM)	Millable Height (CM)	No. of Internodes	Girth of Internodes	Length of Internodes	Weight (Kg)
1.	T.C. Co 86032 (Healthy)	168	270	250	20	10	11	1.530
2.	T.C. VSI 08005 (Healthy)	158	260	235	21	11	13	1.530
3.	T.C. VSI 08005 (Infected)	155	190	170	17	10	12	0.970
4.	MHAT Co 86032 (Healthy)	161	231	210	24	11	10	1.230
5.	MHAT Co 86032 (Infected)	159	225	200	19	10	11	0.970
6.	MHAT VSI 08005 (Healthy)	149	255	235	23	9	9	1.240
7.	MHAT VSI 08005 (Infected)	132	238	210	21	11	10	1.230
8.	Control Co 86032 (Healthy)	153	245	220	23	10	16	1.310
9.	Control Co 86032 (Infected)	145	180	160	18	8	16	0.450
10.	Control VSI 08005 (Healthy)	166	243	220	25	11	15	1.405
11.	Control VSI 08005 (Infected)	165	240	215	25	12	13	1.210

This table presents agronomic yield characteristics of sugarcane under different treatments (Tissue Culture, MHAT, and Control) and two varieties (Co 86032 and VSI 08005), comparing healthy and infected plants for each treatment.

The micro propagated T.C. Co 86032 variety recorded higher mean values for yield components *viz*. total millable canes (168), total height (270 cm), height of millable canes (250 cm), single cane weight (1.530 kg) at harvest when compared to the sett propagated control Co 86032 variety (153, 245 cm, 220 cm, 1.310 kg), respectively (Table 3). T.C. Co 86032 & T.C. VSI 08005 show the best performance in terms of height and weight. Control VSI 08005 also performs well with high internode numbers and good weight. Control Co 86032 (Infected) massive drop in all parameters, least resistant. T.C. treatments also suffer sharp drops in weight and height under infection. MHAT VSI 08005 and Control VSI 08005 maintain relatively stable weights and internode counts even when infected. VSI 08005 variety overall shows stronger resistance than Co 86032. The results of the present study clearly revealed the superiority of micro-propagation planting material over conventional sett propagation.

Table 4: Juice Quality Parameters

Sr. No.	Treatment	Brix	Sucrose	Purity
1.	T.C. Co 86032 (Healthy)	20.89	19.99	95.45
2.	T.C. VSI 08005 (Healthy)	21.22	20.19	95.13
3.	T.C. VSI 08005 (Infected)	20.89	21.15	101.44
4.	MHAT Co 86032 (Healthy)	20.85	19.86	95.26
5.	MHAT Co 86032 (Infected)	22.65	18.94	83.62
6.	MHAT VSI 08005 (Healthy)	21.69	20.11	92.72
7.	MHAT VSI 08005 (Infected)	21.82	20.27	92.97
8.	Control Co 86032 (Healthy)	20.72	20.36	98.26
9.	Control Co 86032 (Infected)	21.29	20.43	95.97
10.	Control VSI 08005 (Healthy)	22.55	20.82	92.35
11.	Control VSI 08005 (Infected)	22.44	21.44	95.41

This table measures Brix, Sucrose content, and Juice Purity in different sugarcane treatments and conditions (healthy vs. infected) across two varieties such as Co 86032 and VSI 08005.

In tissue culture treatment, healthy Co 86032 variety has moderate values across all - Brix (20.89), Sucrose (19.99), Purity (95.45%) and healthy VSI 08005 variety has high Brix and Sucrose (21.22 & 20.19), good Purity (95.13%). T.C. VSI 08005 performs best in juice quality, even when infected. In MHAT treatments healthy Co 86032 variety has balanced profile of Brix (20.85), Sucrose (19.86), Purity (95.26%) and healthy VSI 08005 variety has moderate-high Brix and Sucrose, Purity (92.72%). MHAT is less effective under infection, especially in Co 86032. In control treatments healthy Co 86032 has low Brix but high Sucrose and Purity (98.26%) and in infected Co 86032, slight increase in all metrics, still maintains decent quality. VSI 08005 infected plant retains values well; Sucrose (21.44%) and high Purity (95.41%). Control VSI 08005 is stable under infection and has consistently high juice quality.

Conclusion

Meristem culture demonstrated potential for managing yellow leaf disease in sugarcane, particularly in the Co 86032 genotype. It significantly reduced disease incidence compared to controls. However, the technique's success varies by genotype, and further refinement in meristem culture protocols, along with strict hygiene during propagation and hardening, is recommended to ensure consistent production of disease-free plants.

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