PLANT PATHOLOGY

Technical Programme – 2023-2024

PP 14 : Identification of pathotypes of red rot pathogen

Objective : To gather information on the major pathotypes of red rot from the different areas/zones.

Year of start : 1983-84 (Continuing project)

Location :

North West Zone	:	Lucknow, Shahjahanpur, Kapurthala, Uchani and Karnal (SBI)
North Central Zone	:	Pusa and Seorahi
East Coast Zone	:	Anakapalle and Cuddalore
Peninsular Zone	:	Navsari, Coimbatore and Thiruvalla

Working isolates showing pathogenic variability from the previously reported pathotypes at different centers will be confirmed at the following centers: Lucknow and Karnal (North-West zone) and S.B.I., Coimbatore (Peninsular and East Coast zones). The participating centers will deposit such working isolates at the above mentioned centers latest by June 15 of each year. The zonal centers will also maintain the type cultures.

Sugarcane Differentials (20 Nos.): 1. *Baragua* (*S. officinarum*); 2. *Khakai*(*S. sinense*); 3. SES 594 (*S. spontaneum*); 4. CoS 767; 5. BO 91; 6. CoC 671; 7. Co 7717; 8. Co 997; 9. CoJ 64; 10. Co 1148; 11. Co 419; 12. Co 62399; 13. Co 975; 14. CoS 8436; 15. Co 7805;16. Co 86002;17. Co 86032;18. CoV 92102;19. CoSe 95422 and 20. Co 0238.

No. of isolates: Virulent isolates collected from red rot affected canes of commercially cultivated varieties in the zone.

Method of inoculation: Plug method of inoculation is to be used (Details vide PP.17). Inoculations with each isolate to be done on all the differentials with freshly prepared spore suspension. All inoculations to be completed in 2 days by last week of August.

Observation: One observation at 60th day of inoculation.

Evaluation : The canes are to be split open longitudinally. Inoculated canes free from borer infestation and other damages are taken for evaluation. Based on parameters viz., nodal transgression, lesion width, white spots, top yellowing/drying, rind infection and sporulation over the rind, the host reaction is categorized into three groups viz., Resistant (R), Susceptible (S) and Intermediate (X) as follows –

R **:** Lesion width laterally restricted; nodal transgression up to 2 nodes; white spots, rind infection, sporulation over the rind and yellowing/drying of tops absent.

S **:** Lesion width laterally spreading, nodal transgression more than 2 nodes; white spots progressive or restricted; in case of progressive white spots, rind infection, sporulation over the rind and yellowing/drying of tops absent or present.

X **:** Lesion width laterally restricted or spreading; nodal transgression more than 2 nodes; white spots absent or present (restricted type), rind infection, sporulation over the rind and yellowing/drying of tops absent.

PP 17: Evaluation of zonal varieties for resistance to red rot, smut, wilt, YLD, brown rust and pokkah boeng

Objectives: To gather information on the relative resistance to red rot, smut and wilt of the entries in zonal varietal trial of the respective zones.

PP 17 A RED ROT

Locations:

North West Zone	**:**	Lucknow, Kapurthala, Uchani, Shahjahanpur, Pantnagar and Karnal (SBI)
North Central Zone	**:**	Pusa, Motipur and Seorahi
North East Zone	**:**	Buralikson
East Coast Zone	**:**	Anakapalle and Cuddalore
Peninsular Zone	**:**	Thiruvalla, Navsari and Coimbatore

Year of Start: 1986-87 (Continuing project)

Varieties: All the centres will test all the entries of early and mid-late groups under IVT and AVT of the respective zones. The seed material for this programme is to be obtained from the respective breeders of the centres. One six-meter row of at least 20 clumps may be kept for inoculation with each pathotype by plug/nodal cotton swab method. Any red rot susceptible variety of the same maturity group may be used as standard (check).

Inoculum (Pathotypes to be used):

North West Zone	**:**	CF08 & CF13 (To be inoculated separately)
North Central &		
North Eastern Zones	**:**	CF08& CF13 (To be inoculated separately)
East Coast Zone	**:**	CF06
Peninsular Zone	**:**	CF06 & CF12

(Note: If pathotypes are not available, CF08 and CF12 may be obtained from ICAR-IISR, Lucknow; and CF06 & CF12 from ICAR-SBI, Coimbatore).

Freshly sporulating 7-day-old culture in Petri-dishes will be taken. The conidial mass will be washed with 100 ml of sterile water and collected in a flask. Conidial suspension at a spore concentration of one million conidia per ml will be prepared for inoculation. Fresh inoculum should always be used for inoculation. To maintain the virulence of pathotype, it should be inoculated in a susceptible variety and re-isolated and purified.

Method of inoculation

1. Plug Method: Two canes in each of the 20 clumps to be inoculated. Inoculation is to be done in the middle of the 3rd exposed internode from bottom and two drops of the spore suspension is to be injected with a large syringe in each cane and sealed with plastic clay (plasticine) or modeling clay.

2. Nodal Cotton Swab Method: Two canes in each of 20 clumps will be inoculated by removing leaf sheath (lower most green leaf sheath) and immediately placing cotton swab (dipped in freshly prepared inoculum suspension) around the cane covering nodal region. The cotton swab should be held in place by wrapping parafilm® around the cane stalk.

Evaluation

1. Plug Method: The canes to be split open longitudinally sixty days after inoculation along the point of inoculation. Inoculated canes free from borer infestation and other damages are taken for evaluation. This is graded on the international scale of 0-9 as follows:

Variety (genotype): ---------- Method of inoculation: ----------

No. of canes evaluated	Condition of tops*	Lesion width ** (LW)	White spot ‹ (WS)	Nodal transgression ※ (NT)	Total Score	Remarks
1.						
2. to						
15.						

* 1.Condition of top: Green (G)-0; Yellow (Y)/Dry (D)-1.
**2. Lesion width above to inoculated internode is assigned the score 1, 2 or 3
‹ 3. White spot is assigned score of 1 or 2 according to whether it is restricted or progressive.

※4. N.T. No. of nodes crossed above the inoculated internode and given the score as:

1- if one node crossed; 2-if two nodes crossed; 3. if three nodes are crossed (maximum)
Average Score = Total Score/No. of canes evaluated

Disease reaction: 0-9 scale

0.0 to 2 - R
2.1 to 4 – MR
4.1 to 6 – MS
6.1 to 8 – S
Above 8 – HS

Note: Average score is taken into account for assigning the disease reaction.

2. Nodal Cotton Swab Method: Remove cotton swab and scrap the node with a knife. Record presence/ absence of lesions. In case lesions are progressing into stalk, the reaction is to be recorded as S (susceptible) and if no lesion development, then declare it as R (resistant).

PP 17 B. SMUT

Locations :

North West Zone	**:**	Lucknow, Kapurthala, Uchani, Shahjahanpur and Pantnagar
North Central Zone	**:**	Pusa and Seorahi
East Coast Zone	**:**	Anakapalle and Cuddalore
Peninsular Zone	**:**	Coimbatore, Navsari and Pune

Year of Start: 1994-1995

Varieties: All the entries of early and mid-late group under IVT and AVT of the respective zones. The seed material is to be obtained from the respective breeders of the centre.

Inoculum : *Sporisorium scitamineum* (Syn. *Ustilago scitaminea)* teliospores freshly collected from smut susceptible sugarcane varieties will serve as source of inoculum.

Storage : Freshly collected whips are air dried by keeping under shade and teliospores are collected in butter paper bags and are stored in desiccator under anhydrous calcium chloride. Spore viability is to be ensured before inoculation.

Inoculation : The method of inoculation consists of steeping of setts (three bud) for 30 minutes in a spore suspension of over 90% viability and with a spore load of one million spores per milliliter.

Plot size & Planting : The plot size is one, 3-metre row planted with 10, three-bud setts with a minimum of two replications.

Standards : Any smut susceptible and resistant variety of same maturity group may be used as standard (check).

Observations: Number of smut affected clumps per row are to be recorded. Smut incidence at fortnightly intervals has to be recorded up to harvest of the crop.

Evaluation : Evaluation is based on percentage of total clumps infected (No. of affected clumps/total clumps x100). It is required to maintain at least 15 to 20 clumps in each genotype before arriving at the percentage of infection. The following grading is to be followed for disease reaction:

0 %	**:**	Resistant
>0 to 10 %	**:**	Moderately resistant
>10 to 20 %	**:**	Moderately susceptible
>20 to 30 %	**:**	Susceptible
Above 30 %	**:**	Highly susceptible

PP 17 C. WILT

Location : Kapurthala, Shahjahanpur, Lucknow, Pusa, Navsari, and Anakapalle

Year of Start: 2000-2001

Varieties : Entries of AVT of the respective zones.

The centres may follow plug method or soil inoculation method for evaluating wilt severity in sugarcane clones and may follow the revised 0-9 rating scale to assess the disease severity.

A. Soil borne inoculum (0-9 Scale)

Preparation of *Fusarium sacchari* inoculum for application in soil: Mix 250 g sorghum seed (ground powder) and 750 g sand in 1:3 ratio and add 50-100 ml of distilled water (depending upon the soil moisture) in the container. Put 100 g of sorghum-sand mixture in 250 ml conical flasks and sterilize at 15 lb psi for 2 hr. After 2 days, inoculate each flask with 4-5 mycelia discs of *F. sacchari* grown on oat meal agar medium in a Petri dish and incubate at 22±1°C for 15 days. On 16th day, collect whole inoculum in one tray and mix thoroughly. Apply the inoculum mixture (@100 g/meter row) over the setts uniformly in the furrows at the time of planting.

Evaluation : 0-9 Scale of wilt severity index

Plot size & Planting: Two rows of 5 m length.

Standards (check) : Any wilt susceptible and resistant variety of the zone.

Note: Varieties were screened for wilt resistance in wilt sick plot.

Observations:

1. Germination count at 45 days after planting
2. Appearance of wilt symptoms on the standing canes (on clumps)

3. At the end of 12thmonth, 10 clumps are to be uprooted with roots. All the canes from the clumps will be split open longitudinally and the wiltseverity index scored on a 0-9 scale.

B. Plug Method of inoculation (0-9 Scale*)*

The inoculation is to be carried out on 6-8 months old standing canes. Tencanes per clone are to be inoculated. Inoculation is to be done in the middle of the 3rd exposed internode from bottom and two drops of the conidial suspension is to be delivered onto the bore-hole (as in red rot inoculation) with a large syringe in each cane and sealed with plastic clay (plasticine) or modeling clay.

Evaluation

The canes to be split open longitudinally 60-75 days after inoculation along the point of inoculation. Inoculated canes free from borer infestation and other damages are taken for evaluation. This is graded on the international scale of 0-9 as follows:

Evaluation : 0-9 Scale of wilt severity index

0–2.0 - Resistant (R)
2.1–4.0 - Moderately resistant (MR)
4.1–6.0 - Moderately susceptible (MS)
6.1–8.0 - Susceptible (S)
8.1–9.0 - Highly susceptible (HS).

Wilt severity Grades(0–9 scale)

Grade	Characteristics of key wilt associated parameters in sugarcane
i	**Pith cavities in the internodes above the point of inoculation (0,1,2,3) (Fig. 2a)**
0	No apparent pith cavities
1	Moderate pith cavities occupying entire pith region
2	Cavities along with tissue discoloration covering 2 / 3rd of the internode width
3	Entire internodes are converted into deep pith cavities
ii	**Nodal transgression above inoculated internode (0,1,2,3) (Fig. 2b)**
0	No disease progress in the internodes above the inoculated internode
1	Moderate pith cavities in at least 2 internodes above with moderate tissue discoloration or vascular streaks
2	Moderate to severe cavity formation along with tissue discoloration at least in 4 internodes above
3	Severe cavity formation along with tissue discolorations in more than 4 internodes
iii	**Nodal transgression below inoculated internode (0,1)**
0	No disease progress below the inoculated internodes
1	Moderate to severe cavity formation along with tissue discoloration
iv	**Top dried / green (0,1)**
0	Spindle leaves remain green at the time of examination
1	The spindle leaves show paleness, yellowing, drying or complete death

v	**Stalk external appearance (0,1) (Fig. 2e)**
0	Appears healthy
1	Rind discoloration, total shrinkage of cane/ drying or death of the inoculated canes

*(In case of soil application method, the root health is considered for grading in place of nodal transgression below the inoculated internode; Healthy roots- 0; reduced root volume with infected and discolored roots - 1) *Viswanathan et al. (2021) Modified scale for evaluating sugarcane clones for Fusarium wilt resistance with plug method of inoculation.* ***Sugar Tech*** *https://doi.org/10.1007/s12355-021-01044-9*

Symptoms of wilt displaying different severity grades

a. Pith cavities associated with wilt in sugarcane stalks above the point of inoculation;
0-No disease progress in the internodes above;
1, *F. sacchari* induced mild pith cavities in the upper internodes;
2, tissue discoloration of upper internodes along with pith cavities of medium intensity;
3, all the internodes in the inoculated canes show severe tissue discoloration along with pith cavities. Arrows indicate pathogen inoculated internodes.

b Nodal transgression of *F. sacchari* in sugarcane stalks above the point of inoculation;
0-No disease progress in the internodes above;
1a, *F. sacchari* induced vascular streaks in the upper internodes;
1b, *F. sacchari* induced mild pith cavities in the upper two internodes;

2, *F. sacchari* induced progressive tissue discoloration along with pith cavities of medium intensity in 3–4 upper internodes;
3, the inoculated canes show transgression of the pathogen throughout the canes along with pith severe cavities. Arrows indicate pathogen inoculated internodes.

c. *F. sacchari* spread below the inoculated internode;
0, disease spread above the inoculated internode;
1, disease spread in both the directions from the point of inoculation; Arrows indicate pathogen inoculated internodes.

d. Top dried / green
0- Healthy canopy with green leaves
1 - *F. sacchari* inoculated canes show yellowing of leaves ortotal drying in the canopy

e Stalk external appearance
F. sacchari infection causes paleness and rind discolouration in the affected canes (red arrows) as compared to the healthy canes

The mean wilt severity index is worked out based on the number of canes samples.

$$\text{Mean wilt severity index} : \frac{\text{Sum of wilt indices of individual stalks}}{\text{Number of stalks samples}}$$

Cane sample	Pith cavities (0-3)	Upward Nodal transgression (0-3)	Downward nodal transgression / root health (0-1)	Canopy appearance (0-1)	Stalk appearance (0-1)	Total score	R/MR/ MS/S/HS
1							
2							
3							
4							
5							
Average							

PP17 D: YELLOW LEAF DISEASE (YLD)

Location: Lucknow, Kapurthala, Uchani, Shahjahanpur, Pantnagar, Karnal (SBI), Pusa, Seorahi, Motipur, Buralikson, Anakapalle, Cuddalore, Coimbatore, Thiruvalla, Navsari and Pune.

Year of Start: 2014-15

Varieties : Entries of AVT of the respective zones.

YLD symptoms of mid rib yellowing are expressed during 6-8 months crop stage. If disease severity increases, the yellowing spreads to laminar region and later there will be drying of affected mid rib and adjoining laminar tissue from leaf tip downwards along the mid rib. Another important symptom would be bunching of leaves in the crown. Highly

susceptible variety will exhibit severe foliage drying during maturity stage. In place of yellow discoloration, purple or pinkish purple discoloration may also be seen on the mid rib and lamina. Canes of the affected plant do not dry.

To assess YLD severity, the following disease severity grades are to be given during maturity stages of the crop (3 observations by 8^{th}, 10^{th} and 12^{th}months). Each time, minimum of 25 canes (free from other biotic stresses) are to be scored.

YLD severity grades:

(The colour photographs of YLD symptoms displaying severity grades are available in the soft copy of the technical programme).

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

Mean of the severity grades to be computed and the following YLD severity scale is to be used to assign disease reaction of the variety.

YLD severity scale:

Score	Disease reaction
0.0 - 1.0	Resistant
>1.0 – 2.0	Moderately resistant
>2.0 – 3.0	Moderately susceptible
>3.0 – 4.0	Susceptible
>4.0 – 5.0	Highly susceptible

Symptoms of Yellow Leaf Disease displaying different severity grades

PP17 E: BROWN RUST

Location : Pune, Pravaranagar, Coimbatore, Anakapalle, Navsari

Year of Start: 2020-21

Varieties : Entries of AVT of the respective zones.

Inoculation methodology:Leaf whorl inoculation (for artificial screening)

- As soon as brown rust appears on the check variety (susceptible) in the field, collect rust affected leaves and wash in a beaker of water
- Make suspension of urediniopores in sterilized distilled water (10^4–10^5 spores/ml).
- Pour 1 ml freshly prepared urediniosporesuspension in each leaf whorl.
- Inoculate in 10 clumps (three shoots per clump) of the entries along with susceptible checks.
- Always inoculate in the evening to ensure proper spore germination

Observations:

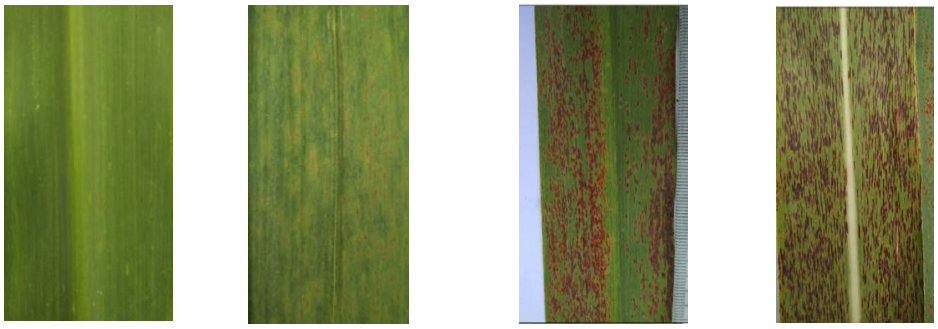
Four weeks after inoculation, record symptoms on leaves by counting-

(i) average number of rust pustules per square inch, and

(ii) number of leaves bearing rust pustules.

Observations under natural conditions:

- The field should be monitored at weekly intervals to record appearance of rust symptoms
- Minimum of five observations need to be taken for calculation of area under disease progress curve.
- Group the entries as R, MR, MS, S based on rust pustule shape, size and appearance.
- According to visual appearance of rust on the foliage, the entry shall be rated as % area infected followed by R / MR/ MS/ S (eg. 10S, 20MR, 50MS, 100S)



R MR MS S

No.	Details	Observation
(i)	Screening under natural / artificial condition	natural / artificial
(ii)	Date of planting	
(iii)	Date of first appearance of rust in the field	
(iv)	Date of artificial inoculation in the entries	
(v)	Date of appearance of symptoms after inoculation	

(vi)	Date of I observation	
(vii)	Date of II observation	
(viii)	Date of III observation	
(ix)	Date of IV observation	
(x)	Date of V observation	
(xi)	Date of VI observation	
(xii)	Date of Final observation	

No	Name of AVT entries	Rust severity at various days of observations						
		I	II	III	IV	V	VI	Final observation
1								
2								
3								
4								
5								

PP17 F: POKKAH BOENG

Location: Lucknow, Kapurthala, Uchani, Shahjahanpur, Pantnagar, Karnal (SBI), Pusa, Seorahi, Motipur, Buralikson, Anakapalle, Cuddalore, Coimbatore, Thiruvalla, Navsari and Pune.

Year of Start: 2020-21

Varieties : Entries of AVT of the respective zones.

(i) Screening:

Symptoms to be observed

Mild- Green plants with pokkah boeng (curling/ twisting of spindle leaves, tearingof leaves, whitish/chlorotic streaks on the leaves) at varying intensities.

Moderate- Yellowing of 3rd/ 4th leaf followed by complete yellowing of foliage and expression of top rot symptom.

Severe- Yellowing of leaves + Discoloration (Light coloured) of stalks + Wilting symptom in opened stalks.

Observe for the presence of above symptoms and grade it as given below:

Varieties*	Per cent infected plants				Disease reaction
	Mild	Moderate	Severe	Total incidence	
V1					
V2					
V3					

- *: No restriction on number of varieties to be studied
- Tag few entries showing PB in early stage and observe for wilt development (pre wilting symptoms) in the same plant.

Disease Reaction:

0-5% - Resistant; 5.0 - 10% - Moderately Susceptible; 10.0 - 20% - Susceptible; > 20% - Highly Susceptible

	Entry	Total plants observed	No. of PB plants	% PB	No. of wilted plants	% Wilt	Both PB +Wilt	% PB +Wilt
V1								
V2								
V3								
V4								

PP 22: Survey of sugarcane diseases naturally occurring in the area on important sugarcane varieties

Objective : To gather information on the diseases naturally occurring in the area on varieties for compiling an all India disease status report yearly

Locations : Lucknow, Kapurthala, Uchani, Shahjahanpur, Pantnagar, Karnal (SBI), Pusa, Seorahi, Motipur, Buralikson, Anakapalle, Cuddalore, Nayagarh, Coimbatore, Mandya, Sankeshwar, Powarkheda, Pravaranagar, Thiruvalla, Padegaon, Kolhapur, Navsari and Pune.

Year of Start: 1989-1990

Observations: Periodic observations in June, September and December in all locations to gather information on the per cent incidence of diseases on all varieties of the area (General survey)

In addition to general survey with disease scenario, damage, varieties affected and impact of disease management measures, the following table need to be filled-in by the centres concerned.

No.	Variety	Red rot	Smut	Wilt	Pokkahboeng	Pineapple disease	Yellow leaf	Grassy shoot	Mosaic	Leaf fleck	Brown rust	Orange rust	Stalk rot	Eye spot	Brown spot	Ring spot	Brown stripe	Yellow spot	Ratoon stunting	Leaf scald	Red stripe	Other diseases
1	V1																					
2	V2																					
3	V3																					
4	V4																					
5	V5																					
6	V6																					

7	V7																				
8	V8																				
9	V9																				
10	V10																				
11	V11																				
12	V12																				
13	V13																				
14	V14																				
15	V15																				
16	V16																				
17	V17																				
18	V18																				
19	V19																				
20	V20																				

Mark '√' for the presence of the disease; '×' for absence of the disease in the region

PP 23: Assessment of elite and ISH genotypes for resistance to red rot

Objective : To gather information on *Saccharum* sp. and elite genotypes for resistance to red rot, so that the resistant genotypes could be used in breeding programme as possible donor for resistance

Locations : Kapurthala, Uchani, Karnal, Shahjahanpur, Lucknow, Pusa, Seorahi, Anakapalle, Cuddalore and Navsari.

No. of genotypes: Director, SBI, Coimbatore may be requested in advance for supply of seed material of the genotypes.

Plot size : One, six metre row of at least 10 clumps

No. of isolates: As indicated in PP 17 experiment.

Method of inoculation : Plug method only.

Inoculum : As per details given under PP 17 (Pathotypes to be inoculated individually only)

Method of evaluation : As per details in PP 17

PP 32 : Management of brown spot disease of sugarcane

Objective : To find out effective method of brown spot management through chemicals.

Locations : Pune

Year of Start: 2015-16

Treatment :

I. Variety : Brown spot susceptible variety CoM 0265 (or local susceptible variety)

II. Fungicides

T.1	- Propiconazole	-	0.1 %
T.2	- Hexaconazole	-	0.1 %
T.3	- Triadimefon	-	0.1 %
T.4	- Mancozeb	-	0.3 %
T.5	- Carbendazim	-	0.1 %
T.6	- Control (Untreated)	-	-

III. Time of application of fungicides: To be applied just after appearance of brown spot lesions followed by two sprays at 15 days interval.

Plot size : 6 x 7 sq. m

Design : RBD

Replications : Three

Observations:

1. Germination %
2. Disease incidence% (No. of clumps showing disease / total no. of clumps x 100)
3. Disease severity (% leaf area covered with brown spot lesions based on observations of 10 leaves per clump; total no. of clumps to be observed at least 10)
4. Cane yield per plot and per hectare
5. Brix, Pol %, Purity and CCS %
6. Cost-benefit ratio

PP 33: Management of yellow leaf disease through meristem culture combined with molecular diagnostics

Objective : To produce sugarcane seed cane free from yellow leaf disease through meristem culture.

Locations : North West Zone :Lucknow, Shahjahanpur, Karnal, Uchani & Pantnagar

North Central Zone : Pusa

Peninsular Zone :Coimbatore, Pune & Navsari

East Coast Zone :Anakapalle&Cuddalore

Year of Start : 2016-17

I. Developing virus-free plants through meristem culture

i. Tissue culture

Methodology :

(i) 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.

(ii) 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.

(iii) 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.

(iv) **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.

(v) **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.

ii. 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
Annealing at 65°C for 1 min } 30 cycles

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.

Observations to be recorded:

S.No.	Name of variety	No. of plantlets during hardening process	No. of plantlets transplanted in field	YLD incidence (%)	
				Breeder Seed crop	Foundation Seed
1					
2					
3					

II. 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).

PP34:	**Efficient delivery of fungicides and other agro inputs to manage major fungal diseases in sugarcane**

PP34a

Objective: To demonstrate efficient delivery of plant protection chemicals or agro inputs/ microbes through mechanized delivery for effective disease management and increased settling vigour.

Locations :

North West Zone : Lucknow, Shahjahanpur, Uchani, Karnal&Kapurthala

North Central zone : Pusa&Seorahi

Peninsular Zone : Coimbatore, Pune & Navsari

East Coast Zone : Anakapalle&Cuddalore

Year of Start : 2020-21

I. Disease management trials

The research work involves sett treatment with fungicides and other inputs in “sett treatment device” (STD) to manage red rot, smut and wilt from primary sources of infection. In the trials, disease affected setts can be used as seed cane for fungicide treatment and tested

against healthy canes. In case infected canes are not available or not sure of sett infections for red rot and wilt, respective pathogen inoculum may be as follow:

Inoculum preparation: One kg of sorghum grain (partially broken grains without powdering) and sand mixture (1:3 ratio) mixed with 100 ml of distilled water. The thoroughly mixed medium is to be distributed in container either in glass bottle or 500 ml conical flask and sterilized at 15 lb pressure for 2 hr. After 2 days, each container is inoculated with mycelia/spore suspension of *C. falcatum* / *F. sacchari*. After 15 days, the inoculum will be ready for application.

Method of application:150 g of grain inoculum/ 20 ft row is applied at the time of planting. The inoculum is to be applied on the setts in the furrows and covered with soil before irrigation and it has to be mixed with equal quantity of sand to have uniform distribution. Alternatively disease affected canes may be chopped and incorporated in the furrows at the time of planting to induce red rot and wilt. For smut, infected setts to be used as seed cane.

A. Management of Red rot

Treatments:

T1 - Sett treatment in STD with fungicide

T2 - Sett treatment in STD with fungicide + Soil drenching by 45^{th}& 90^{th} day

T3 - Infected setts/ Setts + Grain inoculum

T4 - Healthy setts

Fungicide: Thiophanate methyl (Roko 70WP) – 1.3g/ lit (0.1%)

Vacuum level: 200 mmHg

Duration:Vacuum buildup – 5min; Retention – 15min; Air release: 5-10min

B. Management of Smut

Treatments:

T1 - Sett treatment in STD with fungicide

T2 - Sett treatment in STD with fungicide + Spray by 45^{th}& 90^{th} days

T3 - Setts from infected clump

T4 - Healthy setts

Fungicide: Propiconazole (Tilt- 25Ec) – 0.4ml/ lit (100 ppm)

Vacuum level: 200 mmHg

Duration:Vacuum buildup – 5min; Retention – 15min; Air release: 5-10min

C. Management of Wilt

Treatments:

T1 - Sett treatment in STD with fungicide

T2 - Sett treatment in STD with fungicide + Soil drenchingat 45^{th} and 90^{th} days

T3 - Setts from infected clump

T4 - Healthy setts

Fungicide: For sett treatment -Propiconazole (Tilt- 25Ec) – 0.4ml/ lit (100 ppm)

For Soil drenching – Carbendazim (Bavistin 50WP) – 1g/ lit

Vacuum level: 200 mmHg

Duration:Vacuum buildup – 5min; Retention – 15min; Air release: 5-10min

Observations: Per cent germination; Per cent disease/crop survival; Yield attributes

Disease development is to be recorded at pre-emergence as well as post-emergence stages at monthly intervals. Disease development is indicated by death of settlings, yellowing and drying of leaves, mid rib lesions in the whorl and production of dead hearts (red rot/ wilt), that cannot be pulled out easily as in early shoot borer.

II. Delivery of agro inputs to improve settling vigour in nurseries

Treatments

Single bud setts to be treated with micro nutrients, urea, fungicide and insecticide to produce healthy settlings with improved vigour. The centres may plan different treatments with the following ingredients or other inputs depending on their choice. Alternatively liquid culturesof PGPR or Trichoderma can also be treated depending upon their interest. For such treatments a pilot study needs to be conducted to optimize the microbial load for the treatment.

Nutrient mixture: Urea – 0.5g / lit + $ZnSO_4$ – 0.5g/ lit + $FeSO_4$ – 0.5g/ lit

+

Fungicide: Carbendazim –0.5g/ lit (250ppm) (or) Propiconazole – 0.2ml/ lit (50ppm)

+

Insecticide: Fipronil (Regent 5SC) – 0.5ml/ lit (25 ppm)

Vacuum level: 150 mmHg

Duration: Vacuum buildup – 5min; retention – 15min; Air release: 5-10min

Note: If agro-inputs (Nutrient mixture, fungicide and insecticide) used individually, the above concentrations can be doubled.

Observations: Per cent germination; comparative vigour; Yield attributes

PP 34b.

Objective: To demonstrate efficient delivery of plant protection chemicals through drone for effective disease management in sugarcane ecosystem.

Locations :

North West Zone	:	Lucknow, Shahjahanpur, Uchani, Karnal & Kapurthala
North Central zone	:	Pusa &Seorahi
Peninsular Zone	:	Coimbatore, Pune & Navsari
East Coast Zone	:	Anakapalle & Cuddalore

Diseases to be targeted: Red rot, smut, pokkah boeng, foliar diseases

Stages of fungicide application: Three months after planting or ratooning when the crop foliage is not amenable for foliar sprays. The chemicals recommended above. Suitable wetting agents need to be used along with the chemicals.

Timing of sprays: Early morning or later when crop foliage contains enough dew to absorb the fungicides. Spraying of chemicals can also be taken up during evening hours when mild temperature prevails. Conventional high volume sprays may be maintained for first few years to assess comparative field efficacy.

No of sprays: Minimum two

Dosage to be sprayed: The same amount of chemicals recommended in high volume sprays for an unit area.

Observations to be recorded: Disease incidence and intensity before and after fungicide application, rate of disease progress or reduction in comparison to control plots and comparative efficacy to foliar sprays.

PP 35: Development of inoculation techniques for Pokkah boeng disease of sugarcane

Locations :

North West Zone	:	Lucknow, Shahjahanpur, Uchani, Karnal & Kapurthala
North Central zone	:	Pusa & Seorahi
North Eastern zone	:	Buralikson
Peninsular Zone	:	Pune, Thiruvalla and Navsari
East Coast Zone	:	Anakapalle & Cuddalore

Year of Start : 2023-24

Suggested Methods of inoculation to be tested:

A. Sett treatment:

In this method two budded setts of susceptible variety will be dipped in the conidial suspension (concentration 10^6) of pathogen for

15 minutes
20 minutes
25 minutes
30 minutes

Planted in the field and observations were recorded after germination of the crop till the maturity of crop (every after 15 days intervals).

B. Foliar inoculation by spraying:

In this method 4 months old plants of susceptible variety will be selected. The conidial suspension (10^6 conidia/ml) of pathogen will be sprayed on plants apical parts. The spraying of suspension will be made morning and evening for three consecutive days. Five observations will be recorded every after 15 day's intervals.

C. Tooth pick inoculation method:

In this method wooden toothpick will be boiled for 2 hours to remove resin, gums and other types of toxic substances that might inhibit the growth of pathogen. After boiling, they will be washed in tap water and then sun dried. About 10 toothpicks will be placed in 100ml flask having 25 ml of PD broth in it and autoclaved at 15 p.s.i. (Temperature 121^0C) for 20 minutes. Under aseptic condition in Laminar Air Flow, flasks will be inoculated with 5mm disc of 7 days old culture of fungus. Then flasks will kept in BOD incubator at 25±2ºC for 7 days to cover lower 1/3rd part of toothpick by mycelium. After that the canes susceptible variety will be inoculated at the lower 3rd or 4th internode by inserting a toothpick tip overgrown with mycelia of pathogen. The control cane was inoculated with non-infested and sterilized toothpick. Five observations will be recorded every after 15 day's intervals.

D. Cotton swab method:

In the method conidial suspension (10^6 conidia/ml) of the fungus will be prepared to inoculate the canes. Absorbent cotton was dipped in the conidial suspension overnight for the proper soaking of the suspension. Soaked cotton swab will then wrapped around the bud of third inter node of cane from ground level and then sealed with parafilm to maintain the long duration exposure of cane to inoculum.Five observations will be recorded every after 15 day's intervals.

E. Spindle Inoculation:

Conidial suspensions of the pathogen (10^6 conidia/ml) will be dripped into the young spindle (4 to 5 months old plant) of susceptible variety. Five observations will be recorded every after 15 day's intervals.

F. Detached Leaf Assay Inoculation:

In the method at least five leaves will be cut from the susceptible variety with 8 - 10 cm length by sterile scissors. The catted leaves will be inoculated in the laboratory with the conidial suspensions of the pathogen (10^6 conidia/ml). The length of the lesion was measured at 48-h post-inoculation of the said leaves.