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Evaluation of Sugarcane Somaclones for Resistance to Red rot and Yellow Leaf Disease

T M Hemalatha, M Hemanth Kumar and K R Tagore
Agricultural Research Station, Perumallapalle, A. P.

ABSTRACT

Red rot caused by *Colletotrichum falcatum* and Sugarcane yellow leaf disease caused by *Sugarcane yellow leaf virus* are two of the major diseases in sugarcane causing severe losses in susceptible varieties. Twelve promising sugarcane somaclones developed from popularly grown varieties in the zone ie., 2003V46, Co 86032, 2005T16 and two resistant clones (94V101, 97R167) identified for YLD were evaluated for resistance to red rot and yellow leaf disease at Agricultural Research Station, Perumallapalle during 2018-19 and 2019-20 under field conditions. Resistance to red rot disease was studied using four prevalent pathotypes in Andhra Pradesh (Cf 261, Cf 419, Cf 671 and Cf 997) by artificial inoculation *i.e*., Cotton swab and Plug method. The clones were evaluated for YLD based on natural incidence of the disease. Among the twelve clones tested, almost all the clones showed resistant to moderately resistant (MR) reaction to the four red rot pathotypes tested and only one clone, 16T38 showed moderately susceptible reaction to the pathotype, Cf 671. For YLD disease, two prominent clones, 16T7 and 16T37 showed '0' grade for YLD disease with complete absence of symptoms on the leaves and also confirmed by molecular analysis of presence or absence of coat protein of the virus through RT-PCR. The somaclone, 16T7 was found to be best clone showing resistance to both red rot and YLD, highest cane yield, and HR brix%, can be recommended to the sugarcane farmers for cultivation.

Keywords: *RT-PCR, Resistance, red rot, Sugarcane, Somaclones,Yellow leaf disease.*

Sugarcane is one of the major commercial crop playing a vital role in agriculture and industrial economies of the country. It is cultivated in both tropical and subtropical regions in India, and a major source of sugar, khandsari and gur. Diseases are major constraints in the profitable cultivation of sugarcane in India. More than 60 diseases of sugarcane caused by fungi, bacteria, viruses, mycoplasma and nematodes have been reported from different parts of the world. Among the fungal diseases, Red rot, caused by *Colletotrichum falcatum* Went, is an important disease of sugarcane causing the greatest loss to sugarcane industry. Development of new sugarcane cultivars is a continuous process as most of the newly released cultivars to replace the susceptible ones succumb to the pathogen almost as soon as they become popular. This is due to the frequent emergence of new variants of the pathogen. Management of the disease by the use of disease-free seed canes for planting is impractical due to the difficulty in diagnosing the dormant infections of the fungus in seed canes under field conditions (Viswanathan and Samiyappan, 2002).

In recent times, sugarcane yellow leaf disease caused by S*ugarcane yellow leaf virus* (SCYLV) is posing economic threat to the sugarcane industry worldwide. It was first reported in India by Rao *et al.* (2000) and in Andhra Pradesh by Bharati and Kishan Reddy (2007). Disease symptoms are characterized by yellowing of leaf midribs followed by yellowing of the entire leaf blade and internode shortening of green leaf top. Midrib yellowing may be intense or in some varieties may have a reddish tinge and is associated with sucrose accumulation in midribs. This virus is transmitted from plant to plant by aphids in a persistent, circulative, non-replicative manner (Gray and Gildow, 2003) and secondary spread is achieved through aphid vectors (Lehrer *et al*., 2007; Rassaby *et al*., 2004; Scagliusi and Lockhart, 2000). The severity of the disease results in 100% yield losses in case of susceptible varieties.

Since, sugarcane is known to have one of the most complex genomes; breeding for disease resistance in sugarcane through conventional methods is highly impractical. Somaclonal variation has been used by various scientists to recover improved plantlets from a number of genotypes. They may show variation for different parameters like yield, sugar recovery, disease resistance, maturity, drought and salt tolerance. Hence, in the present study, somaclones and their sources varieties were screened for reaction to four isolates of red rot pathogen and yellow leaf disease to identify the resistant genotypes with high yields.

MATERIAL AND METHODS

Field experiments were conducted to evaluate 12 sugarcane somaclones at Agricultural Research

Table 1. Red rot reaction of sugarcane genotypes during 2018-19 and 2019-20.

S. No.	Entry	Reaction of the variety/ genotype to the pathotypes							
		Cf 04		Cf 05		Cf 06		Cf 10	
		Plug method	Cotton Swab method	Plug method	Cotton Swab method	Plug method	Cotton Swab method	Plug method	Cotton Swab method
1	16 T 7	R	R	R	R	MR	R	MR	R
2	16 T 9	MR	R	R	R	R	R	MR	R
3	16 T 10	MR	R	R	R	MR	R	MR	R
4	16 T 12	MR	R	R	R	MR	R	MR	R
5	16 T 14	R	R	R	R	MR	R	MR	R
6	16 T 15	MR	R	R	R	MR	R	MR	R
7	16 T 16	MR	R	R	R	MR	R	MR	R
8	16 T 31	R	R	MR	R	MR	R	MR	R
9	16 T 32	MR	R	R	R	MR	R	MR	R
10	16 T 36	MR	R	MR	R	MS	R	MR	R
11	16 T 37	R	R	R	R	MR	R	R	R
12	16 T 38	R	R	MR	R	MS	R	MR	R
13	2003V46	R	R	R	R	R	R	R	R
14	CO 86032	MS	R	MR	R	MR	R	MR	R
15	2005T16	R	R	R	R	R	R	R	R
16	97R167	R	R	R	R	R	R	R	R
17	94V101	R	R	R	R	R	R	R	R

R- resistant; MR- Moderately resistant; MS- Moderately susceptible

Table 2. Mean YLD severity and disease reaction in somaclones in 2018-19 and 2019-20

S.No.	Variety	YLD	
		Mean YLD severity index	Disease reaction
1	2016 T7	0.0	R
2	2016 T9	1.3	MR
3	2016 T10	1.6	MR
4	2016 T12	1.8	MR
5	2016 T14	2.1	MS
6	2016 T15	1.2	MR
7	2016 T16	1.4	MR
8	2016 T31	2.2	MS
9	2016 T32	0.6	R
10	2016 T36	2.1	MS
11	2016 T37	0.0	R
12	2016 T38	1.4	MR
13	2003V46	4.4	S
14	Co 86032	4.2	MS
15	2005T16	2.1	MR
16	97R167	0.0	R
17	94V101	0.0	R

R- resistant; MR- Moderately resistant; MS- Moderately susceptible; S- Susceptible

Station, Perumallapalle during 2018-19 and 2019-20 for screening against red rot and yellow leaf disease.

Screenings of somaclones against Red rot disease:

Four 3-budded setts/m were planted in six rows of 6 m length at spacing of 0.8 m between two adjacent rows during first week of March, 2018 and 2019. The experiment was laid with single series and was non-replicated. Fertilizers of 224 kg N, 112 kg P_2O_5 and 224 kg K_2O /ha were applied. Two methods of inoculations ie., Plug method and Cotton swab method were followed. Four pathotypes (Cf 04, Cf 05, Cf 06 and Cf 10) prevalent in A. P. were isolated through single spore isolation technique from affected plants/canes.

Plug method

About 40 healthy standing canes in each clone were inoculated on the 3rd internode from the ground with spore suspension of the isolates individually at a concentration of 6 x 10^5 spores/ml (Satyanarayana *et al.*, 1984) when the crop was seven months old during October. Observations on external and internal symptoms were recorded at 60 Days. After Inoculation (DAI) with respect to nature of tops, lesion width, nature of white spot and nodal transgression. The genotypes were classified in to different groups of

resistance or susceptibile based on total average value for the above four characters on 0-9 scale.

Symptom and corresponding score (0-9 Scale)

I) Lesion width:

A) Confided inoculated internode - scale '1'; B) Lesion sharply restricted in width-scale '2' C) Lesion bending to spread laterally- scale '3'.

II) White spots:

A) White spots absent - scale '0'; B) White spots restricted-scale '1'; C) White spots prominent-scale '2'.

III)Nodal transgression:

A) One node crossed- scale '1,; B) Two nodes crossed- scale '2'; C) Three or more nodes crossed-scale '3'.

IV) Condition of tops:

A) Green- scale '0' ; B) Yellow or dry-scale '1'

Categorization based on 0-9 grade scale

0.0 - 0.2 grade- resistant; 2.1 - 4.0 grade - Moderately resistant; 4.1 - 6.0 grade - Moderately susceptible; 6.1 - 8.0 grade - Susceptible; Above 8.0 grade - Highly susceptible .

Cotton swab method

Forty healthy canes were 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 was held in place by wrapping parafilm around the cane stalk. After 60 days, the cotton swab was removed and observed for presence/absence of lesions. In case lesions are progressing into stalk, the reaction was noted as S (susceptible) and if no lesion development, then R (resistant).

Screening for yellow leaf disease

Four 3-budded setts/m were planted in six rows of 6 m length at spacing of 0.8 m between two adjacent rows during first week of March, 2018 and 2019. The experiment was laid in randomized block design with two replications. The fertilizers N, P_2O_5, K_2O at the rate of 224: 112: 224 kg/ha were applied. The test plots were inspected at weekly intervals. The six top leaves from number –3 to + 3 (top visible dew lap as leaf number +1) were inspected for YLD symptoms. The overall yellowing of leaves of all plants in each plot was graded and recorded. The rating result indicated the symptom severity which was present in the majority of the plants in the test plot. The yellow leaf symptoms were graded visually on a scale from 0 to 5 according to ACRIP on sugarcane scale '0': No symptom of the disease, scale '1': Mild yellowing of midrib in one or two leaves, no sign of typical bunching of leaves caused by YLD. scale '2,: Prominent yellowing of midrib on all the leaves in the crown. No bunching of leaves, scale '3': Progress of midrib yellowing to laminar region in the whorl, yellowing on the upper leaf surface, and bunching of leaves, scale '4': Drying of laminar region from leaf tip downwards along the midrib, typical bunching of leaves as a tuft, scale '5': Stunted growth of the cane combined with drying of symptomatic leaves.

YLD severity scale

Based on the mean score of YLD, the clones were categorised into resistant (0.0 - 1.0), moderately resistant(>1.0 – 2.0) , moderately susceptible(>2.0 – 3.0), susceptible (>3.0 – 4.0) and highly susceptible (>4.0 – 5.0).

RESULTS AND DISCUSSION

Screening for Red Rot disease:

Twelve sugarcane somaclones and the five parental checks were tested for red rot reaction against the four pathotypes by plug and cotton swab methods. Among them, ten clones (2016T7, 2016T9, 2016T10, 2016T12, 2016T14, 2016T15, 2016T16, 2016T31, 2016T 32 and 2016T 37) were resistant to moderately resistant in reaction to all the four pathotypes in plug method and resistant reaction in cotton swab method. Two of the clones, i.e., 2016T36 and 2016T38 derived from 97R167 and 2005T16 were moderately susceptible to the pathotype Cf 06 in plug method while remaining clones showed resistant reaction to the four pathotypes tested. (Table.1). Among the parental checks, Co 86032 showed moderately susceptible reaction to Cf 04 while all the four parents (2003V46, 2005T16, 97R167 and 94 V101) showed resistant to moderately resistant reaction to the four pathotypes in both the methods.

Screening for yellow leaf disease

Screening for YLD resistance revealed that two clones ie., 2016T7 and 2016T37 derived from 2003V46 and 97R167 showed mean severity index of " 0" with resistant reaction while the clone 2016T32 showed mean severity index of 0.6 categorized as resistant reaction. The absolute resistance of the clones , 2016T7 and 2016T37 was confirmed by molecular detection of the presence/ absence of the coat protein of the virus by RT-PCR using coat protein specific primers (Data not provided). The somaclones, 2016T14, 2016T31 and 2016T36 showed severity index above 2.0 with moderately susceptible reaction. The

parental checks showed the severity upto 4.4 showing susceptibility towards YLD. Lehrer and Komor (2008) evaluated the sugarcane cultivars in Hawaii based on the mean YLD severity index and classified the cultivars into three categories *i.e.,* ScYLV-susceptible / infected, ScYLV-resistant and intermediately infected cultivars. The clone, 2016T7 developed from the popular variety 2003V 46 occupies the majority of the sugarcane growing area in Andhra Pradesh and the clone, 2016T37 was developed 97R167, showing resistance to Yellow leaf disease. 2016T7 has superior agronomic qualities and can be recommended for cultivation by the farmers for managing these two diseases which are economically important.

CONCLUSION

The clones showing resistance to red rot disease and yellow leaf disease ie., 2016T7 and 2016T37 can be recommended for cultivation in farmers fields. Especially, the clone 2016T7 derived from 2003V46, the popular grown variety can be suggested to the farmers in the YLD affected areas of A.P.

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