Evaluation of Some Brazilian Sugarcane Varieties for Resistance to Leaf Scald Disease Pathogen in Sri Lanka

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ABSTRACT

Identification of resistant parent clones of sugarcane (*Saccharum spp hybrid*) for leaf scald disease caused by *Xanthomonas albilianeans* is required to produce resistant varieties for commercial cultivation. The present study investigates the resistance of some Brazillian sugarcane varieties against leaf scald disease. Three-budded setts of eleven sugarcane varieties imported from Brazil with wide genetic variation along with the standard varieties were planted in one-metre plots in three replicates in Randomised Complete Block Design to evaluate their reaction to sugarcane leaf scald disease. Three-month old plants were inoculated with the inoculum extracted from the leaf tissue of the diseased plants mixed with leaf scald disease-causing bacterial broth culture according to the standard "Aluminium cap" technique. The disease incidence was recorded at one-month intervals from 30 days after inoculation of the bacterium up to ten months. The percentage disease incidence was calculated and the ratings of the resistance levels were assigned for the test varieties based on the ratings of the standard varieties. The results revealed that the variety SP 89 1115 was resistant to leaf scald disease and while the others were susceptible to the disease. This variety could be used for sugarcane crop improvement program to improve the resistance of the varieties to leaf scald disease in sugarcane.

Keywords: Leaf scald disease, Resistance, Sri Lanka, Sugarcane

INTRODUCTION

Leaf Scald Disease (LSD) is one of the two major bacterial diseases in almost all sugarcane-growing areas in the world including Sri Lanka. The disease is caused by a gram negative bacterium, *Xanthomonas albilianeans* (Ashby, 1929 and Dowson, 1943). It colonises in the vascular system of leaves, stems and roots, and has a significant effect on reducing cane yield (Pan *et al.*, 2004) and the quality of juice (Martin and Robinson, 1961).This disease can cause extensive yield losses in highly susceptible varieties through death of stalks and poor ratooning. It has caused severe losses in *Saccharum offinarum* L. cultivars that were grown in the world until the early part of the twentieth century. The losses have been reduced after the introduction of inter-specific hybrids of *Saccharum* that were resistant to LSD (Hoy and Grisham, 1994).

A great concern with this disease is that the bacterial pathogens can exist for extended period of time in seemingly healthy plants. Since sugarcane is a vegetatively-propagated crop, the pathogens can be readily spread between fields, regions and even countries (Pan *et al.*, 2004). The first documented note on this disease in Sri Lanka was from Kantale, Gal Oya and Walawe in 1961(Egan, 1961).

As the LSD can cause complete crop loss in highly susceptible varieties (Rott and Devis, 2000), breeding for resistant varieties has been the most-adopted strategy for managing the disease. In order to facilitate the directional breeding for LSD resistance, the screening of the sugarcane varieties for their

reactions to this disease is of great importance to avoid crosses between susceptible parent clones to reduce the number of susceptible clones coming through the crop improvement program. This is useful to avoid the releasing of disease susceptible/less tolerant varieties into the commercial plantations, and thereby, to reduce the disease in sugarcane plantations. In this study, an assessment of the reaction of some imported sugarcane varieties from Brazil to LSD was made to identify the resistant/tolerant parent clones to recommend them for sugarcane crop improvement for LSD.

MATERIALS AND METHODS

A field experiment was carried out at the research farm of the Sugarcane Research Institute, Uda Walawe, where the annual average rainfall is about 1450 mm during 2009 - 2010 using the varieties, SP 83 2847, SP 85 3877, SP 86 155, SP 87 365, SP 87 369, SP 89 1115, SP 90 1107, SP 90 1638, SP 90 3414, SP 90 3723 and SP 91 1047 imported from Brazil with a wide genetic variation. Three-budded setts of these varieties were planted along with the standard varieties, namely, Co 997, Triton, Trojan, Co 740, Q 68, Co 775 and Co1001 in one-metre row plots in three replicates in Randomised Complete Block Design to evaluate the resistance of the varieties to LSD.

To inoculate the plants, the bacterium was isolated from the infected leaves with white/cream colour streaks on Wilbrinks medium with slight modifications proposed by Davis *et al.*(1994). The medium contains Sucrose 10.0 g, peptone 5.0 g, yeast extract 5.0 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.25 g, Na_2SO_3 0.05 g, Agar 15.0g distilled water 1 L supplement with KBr (5 g/L),Cephalexin (25 mg/L) and Nivobiocin (30 mg/L).The leaf segments were surface sterilised in 0.1 % cholox for 1 min, washed twice in sterile distilled water. They were transferred to 70 % alcohol for 30 seconds and again washed twice with sterile distilled water. The swabbed leaf segments were transferred to the medium and incubated for 144 hours at 28°C. Single colonies were then serially sub cultured on respective medium to get pure cultures. After getting pure cultures, the bacterium was transferred to Wilbrink broth. The bacterium in 250ml of broth was cultured for two days under shake flask culture-shaken for two days at 150 rpm. This broth culture was mixed with 500 ml of the inoculum which was freshly prepared by grinding disease symptoms bearing leaves. Three-month old randomly-selected ten plants from each variety were decapitated and inoculated with 5ml of the above suspension according to the standard "Aluminium cap" technique (Koike, 1965).

The disease incidence was recorded at one-month intervals from 30 days after inoculation of the bacterium up to ten months. Then the average observed disease percentage was obtained for each variety by calculating the arithmetic average of the percentage disease incidence measured in three replicates. Then a calibration curve was drawn and its equation was derived on the average observed disease incidence (Y) and the previously-assigned leaf scald disease ratings for the standard varieties (X) (SRI, 2009). A graph was also drawn to compare the behaviour of the standard varieties according to their known rating and the behaviour of the same standard varieties based on the field observations. By referring the derived calibration curve, disease ratings for the test varieties were obtained based on their observed disease incidences.

RESULTS AND DISCUSSION

The calibration curve derived using the standard varieties is shown in Figure 1.The equation derived for the calibration curve was $Y = 4.822 - 3.5510X + 1.529X^2$; ($R^2$=98 %) where Y is the percentage disease incidence observed and X is the leaf scald disease rating previously assigned.

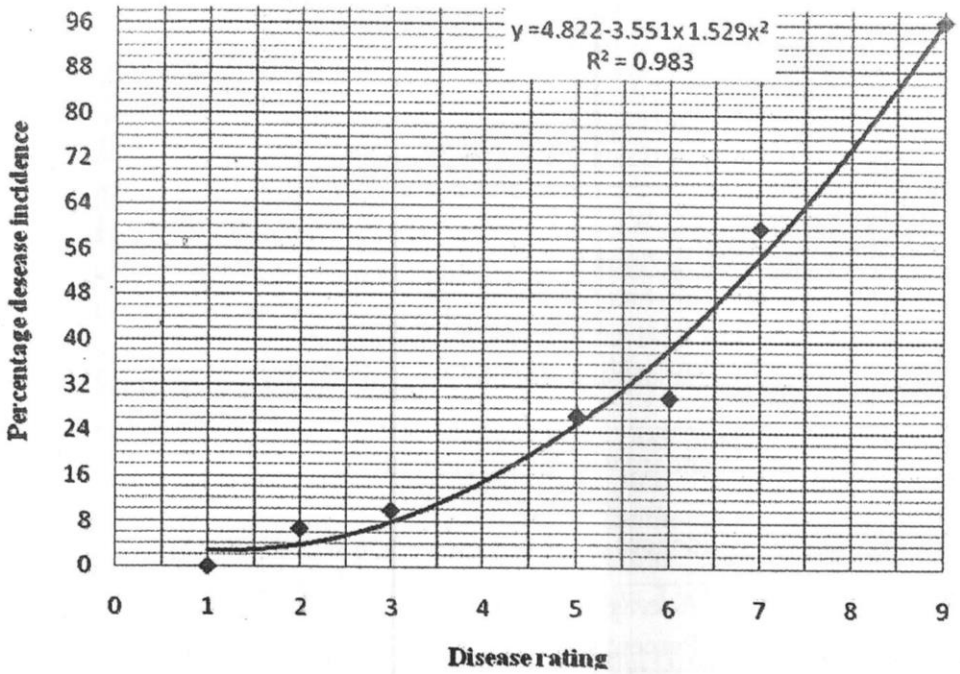

Figure 1 The calibration curve established for leaf scald disease

The currently-used standard varieties and their observed and estimated disease incidences and the estimated ratings based on the calibration equation are summarised in Table 1.

The behaviour of the standard varieties according to their known ratings and that of the same standard varieties based on the field observations are shown in Figure 2.

According to the results, all the standard varieties have shown previously proven disease ratings. There were no much deviations of the standard varieties from their normal behaviour. Therefore, all the test varieties also would have shown their correct reaction to leaf scald disease.

The ratings assigned to each test variety based on their observed disease percentages are summarised in Table 2.

Table 1 The observed and estimated disease incidences and the estimated ratings of standard varieties

Standard variety	Disease percentage	Known rating	Disease status	Observed incidence	Estimated incidence	Estimated rating
Co 997	1.00-3.00	1	HR	0.00	2.84	1
Triton	4.00-6.00	2	R	6.67	3.85	2
Trojan	7.00-9.00	3	R	10.00	7.90	3
Co 740	13.00-25.00	5	MS	26.67	25.12	5
Q 68	26.00-35.00	6	S	30.00	38.29	6
Co 775	36.00-50.00	7	S	60.00	54.50	7
Co 1001	66.00- 100.00	9	HS	96.67	96.04	9

Note: HR- Highly Resistant, R-Resistant, MS-Moderately Susceptible, S-Susceptible, HS - Highly Susceptible

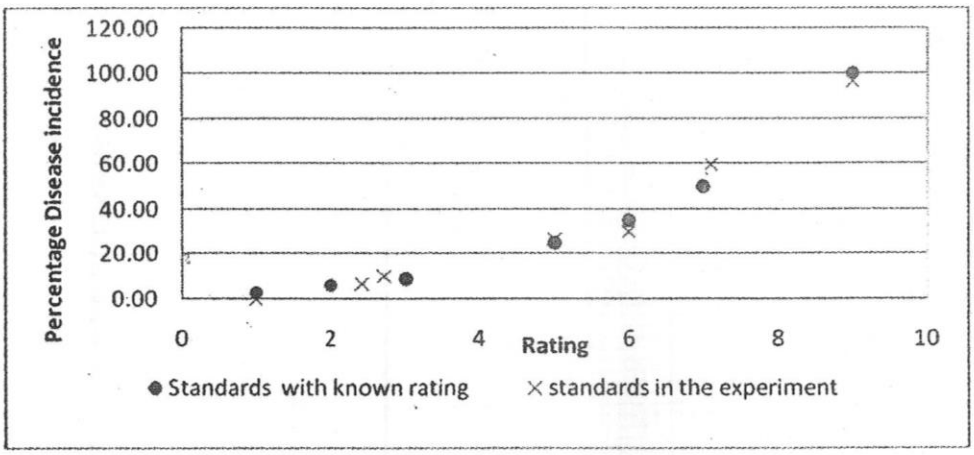

Figure 2 The behaviour of standard varieties in the field

Table 2 Average disease incidences of the test varieties and their ratings

Test variety	Average Disease Percentage	Rating	Disease status
SP 83 2847	82.96	8	Susceptible
SP 85 3877	63.33	7	Susceptible
SP 86 155	100.00	9	Highly susceptible
SP 87 365	53.33	7	Susceptible
SP 87 369	100.00	9	Highly susceptible
SP 89 1115	20.00	4	Resistant
SP 90 1107	93.33	9	Highly susceptible
SP 90 1638	66.67	8	Susceptible
SP 90 3414	53.33	7	Susceptible
SP 90 3723	80.00	8	Susceptible
SP 91 1047	60.00	7	Susceptible

The behaviour of the test varieties and the standard varieties during the experimental period is graphically shown in Figure 3.

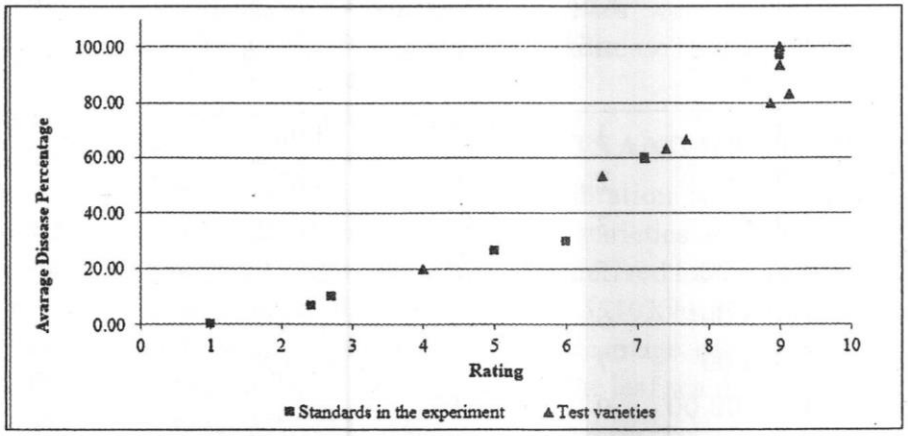

Figure 3 The Behaviour of test varieties and standard varieties in the field

No any test variety has shown a rating lower than 5. Only the variety SP 89 1115 has shown rating of 4, i.e., resistant to LSD. It has recorded a 20 % disease incidence. Furthermore, this variety has showed the same result (a resistant variety) in the gemplasm screening trials in Brazil (Gutierrez and Hoy, 2013) also. All the test varieties, except the variety SP 89 1115, have shown more than 20% disease level during the experimental period. Therefore, those varieties are considered as susceptible to leaf scald disease caused by the local strains of *X. abilianus*.

Conclusion

Based on the results of this study, it can be concluded that the variety SP 89 1115 could be utilised for sugarcane crop breeding program to improve the resistance of the sugarcane varieties to leaf scald disease.

ACKNOWLEDGEMENTS

Authors are thankful to Mr. M.R. Premalal, Lab/Field Attendant in the Division of Crop Protection to support in the field activities. Our sincere gratitude is also goes to Dr. A P Keerthipala, Director/CEO for his valuable suggestions.

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