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Standardization of methodology for screening sugarcane genotypes for resistance to brown rust (*Puccinia melanocephala*)

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Abstract

Two inoculation techniques *viz.*, clip inoculation in leaf whorl with rusted leaf clips and inoculation of rust urediniospores suspension in leaf whorl were evaluated during 2013-14 and 2017-18 for identification of brown rust resistance sugarcane cultivars. The results showed that most severe brown rust symptoms were observed using leaf whorl inoculation method containing inoculum suspension of 10^4 - 10^5 urediniospores /ml. In the leaf whorl inoculation method, higher average no. of rust pustules (27.96 /inch²) and higher no. of leaves bearing rust pustules (8.8) was recorded as compared to the clip inoculation method (21.22 /inch² and 8.26 respectively). This indicates that the leaf whorl inoculation method is better for screening sugarcane genotypes against brown rust than the clip inoculation method. The leaf whorl inoculation technique enabled rapid screening of a large number of cultivars in field using a small amount of inoculums.

Keywords: Sugarcane, *Saccharum officinarum* L., Brown rust, *Puccinia melanocephala*

Introduction

Brown rust caused by *Puccinia melanocephala* H. & P. Syd. Was first reported on sugarcane in 1949 in the Deccan area in India (Patel *et al.*, 1950) [5]. Rust is an economically important disease that results in severe reductions in yield, with losses as much as 40-50% in susceptible varieties (Bailey, 2004 and Rott *et al.*, 2013) [1, 7]. Cultivar resistance is the best means of controlling the disease (Huang *et al.*, 2018) [3]. Natural infection has been the primary means of assessing resistance in sugarcane cultivars against rusts; unfortunately, natural infection is not always efficient in identifying resistant cultivars due to variable environmental conditions. The frequent applications of fungicide and low net profit preclude their use for controlling sugarcane brown rust (Jiang, 1985) [4], making cultivar resistance and cultural practices the most viable alternatives. This means that an effective method for screening genotype reactions to rust pathogens is an important priority. Therefore, a more reliable screening method is needed to effectively select resistant genotypes. With this view, two inoculation techniques *viz.*, clip inoculation in leaf whorl with rusted leaf clips and inoculation of rust urediniospores suspension in leaf whorl were evaluated in the field for identification of brown rust resistance in sugarcane cultivars.

Materials and Methods

Two inoculation techniques *viz.*, clip inoculation in leaf whorl with rusted leaf clips and inoculation of rust urediniospores suspension in leaf whorl were evaluated during 2013-14 and 2017-18 for identification of brown rust resistance in sugarcane cultivars.

Inoculation Methodology:

1. Clip Inoculation in Leaf Whorl

As soon as brown rust appeared in the field, rust affected leaves were selected and leaf bits (clips) measuring 8-10 cm were prepared. Ten rust free plants of the susceptible variety Co VSI 9805 were selected in different location. In 3 shoots of each plant (clump), 2-3 clips were inserted in the leaf whorl of each shoot.

2. Leaf Whorl Inoculation

As soon as brown rust appeared in the field, rust affected leaves of the susceptible variety Co VSI 9805 were selected. Suspension of urediniospores (10^4 - 10^5 spores/ml) was prepared in

sterilized distilled water and 1 ml freshly prepared suspension was poured in each leaf whorl. A total of 10 clumps were inoculated @ 3 shoots per clump.

The inoculated plants were marked by cutting 1/3rd of the tips of the uppermost leaves to identify them for recording observations. Symptoms appeared on leaves of the susceptible variety Co VSI 9805 as a band of pustules after they emerged from the whorl. Disease assessment started 1 week after inoculation and continued at weekly interval for 4 weeks. After four weeks, the observations on average number of rust pustules per square inch and number of leaves bearing rust pustules were recorded.

Results and Discussion

The results are presented in Table 1. The data (2013-14 and 2017-18) on clip inoculation in leaf whorl with rusted leaf clips and inoculation of rust urediniospores suspension in leaf whorl showed that in the leaf whorl inoculation method, higher average no. of rust pustules (27.96 /inch²) and higher no. of leaves bearing rust pustules (8.8) was recorded as

compared to the clip inoculation method (21.22 /inch² and 8.26 respectively). The results showed that most severe brown rust symptoms were observed using leaf whorl inoculation method containing inoculum suspension of 10⁴-10⁵ urediniospores/ml. This indicates that the leaf whorl inoculation method is better for screening sugarcane genotypes against brown rust than the clip inoculation method. Sood *et al.* (2009) [8] reported that the leaf whorl inoculation technique is very useful, reproducible, and efficient for screening a large number of sugarcane cultivars for brown rust and orange rust resistance in field conditions using inoculums containing 10⁵ and 10⁴ urediniospores/ml, respectively. Petersen (1959) [6] also observed an increase in pustule production with increasing density of urediniospores of *Puccinia graminis* f. sp. *tritici* and found that the total number of pustules produced reached a plateau when spore density reached 4×10⁴ spores/ml. Moreover, Gilles and Kennedy (2003) [2] also observed an increase in rust pustule production with increasing inoculums density.

Table 1: Evaluation of inoculation methods for screening sugarcane genotypes against brown rust (2013-14 and 2017-18)

Sr. No.	Inoculation Methodology	Average no. of rust pustules/inch ²						No. of leaves bearing rust pustules					
		2013-14	2014-15	2015-16	2016-17	2017-18	Mean	2013-14	2014-15	2015-16	2016-17	2017-18	Mean
1.	Clip Inoculation in Leaf Whorl	8.33	19.76	26.08	25.85	26.12	21.22	7.6	10.6	9.2	5.4	8.5	8.26
2.	Leaf Whorl Inoculation	10.46	21.86	38.34	31.91	37.24	27.96	7.7	11.6	9.4	5.6	9.7	8.8

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