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SHORT COMMUNICATION

Cut leaf assay: A rapid method for screening rust resistance in sugarcane

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Abstract

Sugarcane rust incited by *Puccinia melanocephala* (brown rust) and *P. kuehnii* (orange rust) becomes very severe under warm, moist climatic conditions. Though there were methods to screen rust resistance under artificial conditions where rust is prevalent, there is no method to screen the rust resistance in a place where rust occurrence is not regular and severe. To overcome this problem, a simple method was devised to screen sugarcane leaves challenged with rust uredospores and maintaining the setup under optimum relative humidity and temperature for 15-20 days for identifying the rust resistant sugarcane clones. This method of rust screening is simple and easy, fast and reliable and many clones can be screened in short period of 20 days.

Keywords: Sugarcane; Rusts; Cut leaf method; Disease resistance

Introduction

In India, sugarcane is grown in about 5 mha area covering 2% share of the total area under major crops. The continuous release of many high-yielding, superior sugarcane varieties adapted to many sugarcane growing regions has brought substantial improvement and revolutionized sugarcane cultivation in the tropical and subtropical parts of India. Under changing climate, the crop is exposed to various biotic and abiotic factors.

Diseases are one of the major constraints in obtaining higher productivity in sugarcane and more than 55 diseases have been reported from India (Rao et al. 2002). Sugarcane rust is an important fungal disease distributed worldwide in more than 60 countries and has economic losses to sugarcane cultivated in several countries (EPPO, 2019). The rusts are obligate pathogens and the wind-born nature of uredospores play a major role in spreading the rust diseases across continents. The continuous presence of sugarcane in the fields as the main crop or ratoon, growing single susceptible varieties over a larger area and conducive environmental factors for the pathogens favour the build of rust inoculum and the development of disease epidemics (Selvakumar and Viswanathan, 2019).

Identification of rust-resistant lines in the field is cumbersome as the disease development is highly influenced by the environment. Various methods and scales have been standardized for screening for rust resistance in the field. Liu (1980) in Puerto Rico, evaluated the commercial varieties and promising lines for brown rust resistance under artificial inoculations. Wrapping the young shoot with rust-affected leaves was found to be better than spraying and dusting uredospores. In Florida, the leaf whorl inoculation technique was standardized and uredospores @10^5 per ml in whorls were introduced and symptoms were observed 30 days after inoculation at weekly intervals. The disease rating scale of 0-4 was followed where 0 and 1 were resistant and 2 was moderately resistant and 3 and 4 were considered as susceptible. Following the criteria, the sugarcane clones are screened for orange rust resistance following the leaf whorl inoculation

technique under field conditions (Sood et al. 2013). In China, Wang et al. (2013) screened the germplasm after spraying the field with uredospores suspension and observed for symptoms after 4-5 weeks using a 0-9 scale. In India, under the All India Coordinated Research project (Sugarcane) natural rust screening was conducted at Padegaon, Pune, Sankeshwar and Anakapalle to screen zonal varieties for rust resistance. In Peninsular India, screening of 275 sugarcane parental clones for rust resistance under natural conditions at Coimbatore revealed that ~60% of clones remained free from rust and 20% exhibited moderately resistant (MR) reactions. Of the 20% susceptible group, ~13% were moderately susceptible (MS) and 7% were susceptible (Selvakumar et al. 2018).

Though there were methods to screen rust resistance under artificial conditions where rust is prevalent, there is no method to screen the rust resistance in a place where rust occurrence is not regular and severe. Under these circumstances, a simple method was devised to screen sugarcane leaves challenged with rust uredospores and maintain the setup under optimum relative humidity and temperature for 15-20 days to identify the rust resistance level.

Collection and preparation of rust inoculum

The leaves of sugarcane cultivars exhibiting rust symptoms with uredospores were collected from the fields at ICAR Sugarcane Breeding Institute. Uredospores were harvested from the leaves in the laboratory by vacuuming the lower side of collected leaves with a glass spore collector attached to a vacuum pump. The freshly collected uredospores were air dried overnight (12-14 hrs) in the dark room temperature (21±25ºC). The collected rust spores were mixed in 100 ml of deionized water to a final concentration of 10^5 uredospores per ml of water. The spore culture was transferred to a hand sprayer. The spray volume of the hand sprayer was approximately 1ml per stroke.

Selection of leaf material for rust screening

The second top visible green leaves free from any visible disease symptoms from sugarcane clones were collected from the field, labelled and brought to the laboratory before drying. The fresh green leaves were cleaned by rinsing with deionized water in the laboratory. Then the surface area was cleaned with 70% ethanol and left aside for 10 minutes. The leaves were cut into three pieces of 15 cm length bits and inoculation with the rust spores.

The leaf bits were kept spread in a closed chamber and were sprayed with the hand sprayer containing uredospores @10^5 spores /ml of water from the height of 30 cm for spores to settle on the leaf surface. Both the sides of leaf surfaces were sprayed and care was taken not to give water spray excessively. After inoculation with the rust pathogen, leaf bits were transferred to a plastic, sterile transparent cylindrical jar (20 cm height and 8 cm diameter) containing 100 ml of sterile deionized water (Fig. 1).

The leaf bits were kept erect with lower leaf section immersed in the cooled boiled water up to 1cm. Three leaf bits were used per treatment in each container. One leaf bit with the same size (15 cm height) was kept as untreated control. The jars were covered with perforated lids to avoid water droplet condensation inside the jar. Then, the jars were maintained in a plant growth chamber at 22±0.5ºC for 24 hours followed by 8 hrs of fluorescent light at

Figure 1. Leaf bit assay for rust screening inside plant growth chamber

22±0.5ºC and 16 hrs dark at 22±0.5ºC during the experimentation period. The symptom of rust development was observed starting 5 days after exposure to rust spores and up to two weeks. The degree of rust infection on the leaf sections will be scored and analyzed by scientists using a (AICRP) scale for rust screening and grouped as resistant and susceptible based on rust symptoms expressed on leaves. The information gathered from these evaluations can be utilized to measure the degree of rust resistance of various sugarcane cultivars. For choosing and breeding sugarcane cultivars that are more resistant to rust infections, this knowledge is useful.

The 'cut leaf assay' has a variety of benefits, including the ability to assess numerous plant samples simultaneously and a relatively short turnaround time. This approach is especially helpful in the early stages of plant breeding programmes, when scientists must swiftly pinpoint rust-resistant cultivars that show promise for further study and improvement. The early detection of rust pathogens in the field becomes a reality with the integration of conventional and molecular techniques and helps in achieving immediate control through fungicides.

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