

Full Length Research Paper

Standardization of inoculation technique of sugarcane smut (*Ustilago scitaminea*) for evaluation of resistance

Paramdeep Singh, Bipen Kumar, Ritu Rani* and Madhu Meeta Jindal

Department of Plant Pathology, Punjab Agricultural University, Ludhiana- 141004, India.

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Different inoculation techniques *viz.* inoculation of sets by dipping in spore suspension, bud inoculation with hypodermic syringe, bud wrapping by cotton swab dipped in smut suspension and inoculation of underground bud at the time of tillering (end May) were tested to screen against sugarcane smut in the field as well as in the laboratory conditions. In the field, out of the tested inoculation techniques, the maximum disease incidence (60.63%) was observed when buds were inoculated with hypodermic syringe and minimum (8.55%) in inoculation of underground buds at the time of tillering stage. The result showed that inoculation through mechanical injury significantly increase disease incidence but at the same time also affect the bud germination (15.56%). Significant difference in smut incidence was also observed when the inoculation was carried by teliospores and sporidia separately.

Key words: *Ustilago scitaminea*, inoculation technique, screening, resistance, sugarcane.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is attacked by more than two hundred and forty diseases caused by fungi, bacteria, viruses, phytoplasmas and nematodes in India (Rott et al., 2000). Of these, fungal disease like red rot, smut and wilt are of major importance and are mainly responsible for reducing the yield and deterioration of the juice quality (Chona, 1956; Waraitch and Kumar, 1984). Sugarcane smut caused by *Ustilago scitaminea* Sydow is considered as an important disease next to red rot. The basidiomycetous fungus belongs to the order Ustilaginales and family Ustilaginaceae first described in 1870 as *Ustilago sacchari* by Mundkur (1939). Sydow (1924) opined that the smut fungus of sugarcane is distinct from *U. sacchari* and called it *U. scitaminea*. As the pathogen

attacks only the meristematic tissues, it is generally referred to as a primitive parasite and it is main problem of tropical India, but now it is also becoming a problem on some varieties in North India. The disease is prevalent in all the countries that lie between 20° N and 20° S (Martin et al., 1961). The pathogen produces abundant tiny brownish black, echinulate, spherical spores ranging from 5.2 to 8.5 µm in diameter. Teliospores of *U. scitaminea* are shed from the whip and disseminated through the wind. The maximum dispersal of spores occurred at 24-27°C and 50-60 percent relative humidity (RH). The characteristic symptoms of the smut are the dark brown, whip-like fungal sorus that develops from the apex of infected stem (Butler, 1918). The wind borne spores are

*Corresponding author. E-mail: ritubansalpau@gmail.com.

spread in the standing cane fields and can infect newly planted setts in the soil. The infection take place through the buds that may soon develop into whips; but the mycelia may remain dormant, and the use of such infected stalks as seed cane spread the disease. Whip development is determined by the season as well as the age and physiological condition of the crop. In India, many superior varieties such as CoS 510, Co 419, Co 453, Co 740, Co 975, Co 1158, Co 62175 and Bo11 have gone out of cultivation due to attack of this disease. The first epidemic of smut occurred during 1942-43 in Bihar and affected 66% of cane area (Chona, 1956; Alexander, 1986). In Karnataka during 1947-48, smut severity was so high, particularly on Co 419 that the disease had to be contained by banning ratoons crop (Subramanian and Rao, 1951). Currently, the disease has established in all the sugarcane growing states of the country especially Maharashtra, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Bihar and Orissa. Due to free movement of sugarcane from the neighboring states to Punjab, the disease incidence up to 0.8% was noticed in year 2006 (Anonymous, 2007). However, the risk of smut incursion in the state is high as un-recommended varieties grown in the state are susceptible to the disease.

Among the management strategies the best option for long term control of smut is the use of resistant cultivars. Traditional method of screening sugarcane cultivars for resistance to smut is time consuming, require large area and result are available after a long period (6-18 months). Thus, evaluation for smut resistance is commonly delayed and at the end of breeding cycle which is usually of 6-8 years, only few selections are left. A simple and rapid screening method, therefore, is necessary for evaluation of large number of progenies during preliminary selection cycles of a breeding program to expedite the development of smut resistant genotypes.

MATERIALS AND METHODS

Testing of different inoculation techniques of sugarcane smut

Field evaluation

Field experiment on testing of four inoculation techniques with two types of spores (teliospores and sporidia) of *U. scitaminea* was conducted at University Seed Farm, Ladhowal, Punjab Agricultural University, Ludhiana, India.

One hundred and thirty five single budded sets of mid-season sugarcane smut susceptible variety CoJ 88 were used for each method of inoculation. Planting of sets was carried in three rows of 4.0 m length at 0.75 cm depth in each plot. Three replications were maintained for each treatment.

Two types of smut spores (teliospores and sporidia, $1 \times 10^6/\text{mL}^{-1}$) were used for inoculation. A suspension of freshly collected teliospores was made in sterile water. Viability of the smut spores was tested and a collection showing a viability of more than 70% was used for the inoculation. For culturing of smut sporidia, teliospores collected from infected canes were dusted on Potato Dextrose Agar medium (Peeled Potato- 250 g, Dextrose- 20 g, Agar-agar powder- 20 g and 1000 mL distilled water) in test tubes

and incubated for 10 days at $22 \pm 2^\circ\text{C}$. After 10 days of incubation, sporidia were collected and desired concentration of sporidia (1×10^6 spores/ mL^{-1}) was prepared by using sterile distilled water and used for inoculation studies as follows:

1. Inoculation of setts by dipping in spore suspension: Single budded sets of sugarcane variety CoJ 88 were inoculated by dipping in teliospores/sporidia suspension for 30 min and incubated for 24 h before sowing.
2. Bud inoculation with hypodermic syringe: Cane buds were inoculated with teliospores/sporidia with the help of hypodermic syringe. Each bud was injected with 0.5 mL spore suspension (1×10^6 spores/ mL^{-1}).
3. Bud wrapping by cotton swab dipped in smut suspension: A thick spore suspension (5×10^6 spore/ mL^{-1}) was prepared and applied with cotton swab on the buds.
4. Inoculation of underground bud at the time of tillering (ended May): Tillering was started in the last week of May and soil around the germinated clumps was carefully dug out. Four to five basal leaves of mother shoot were removed carefully to expose the young buds unhurt and 2-3 buds were inoculated by painting the inoculum on them with the help of hairbrush. Inoculations were carried in the evening to avoid drying. Immediately after inoculation, soil around the clumps was filled and pressed slightly to avoid any uprooting of young cane plant. After the inoculation, the field was irrigated.

Germination of setts was recorded after 30 and 45 days of planting. Observations on smut incidence were recorded fortnightly starting from 1st week of June till harvesting of the crop. Rogueing of the disease clumps was done at each observation. Cumulative smut infection for the whole season was calculated as per the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected clumps in a treatment}}{\text{Total number of germinated clump in a treatment}} \times 100$$

Laboratory evaluation

In vitro tissue culture raised young plants of a variety CoJ 88 were procured from Biotechnology Section of the Department of Plant Breeding, Genetics and Biotechnology. Plantlets were scrapped by scalper and then the plantlets were inoculated with teliospores and sporidia (1×10^6 spore/ mL^{-1}) separately with the help of hairbrush. Inoculated plantlets were transformed in a long jar containing MS medium (Stock no. I- 50 ml; Stock no. II- 20 ml; Stock no. III- 20 ml; Stock no. IV-10 ml; Stock no. V - 10 ml; Sucrose- 30 g; Anasitol - 100 mg; Sterilized water 1000 mL). Plantlets were inoculated with 0.5 μL sterile distilled water served as control. All plantlets were maintained in growth room at $25 \pm 1^\circ\text{C}$ and observed regularly up to three months for smut sori (sorus like spore mass of the fungus) development.

RESULTS AND DISCUSSION

Testing of different inoculation techniques for sugarcane smut

Field evaluation

Out of four inoculation techniques, inoculation of sets by dipping in spores suspension gave maximum sett germination of 77.03 and 76.66% after 30 and 45 days of sowing respectively (Table 1). The minimum set germination of 62.46 and 64 .81% was observed after 30

Table 1. Effect of different smut inoculation techniques on sett germination

Inoculation technique	Per cent sett germination					
	After 30 days			After 45 days		
	By Teliospores	By Sporidia	Mean	By Teliospores	By Sporidia	Mean
Inoculation of setts by dipping in spore suspension	76.29* (60.87)	77.77 (61.86)	77.03 (61.37)	77.03 (61.34)	76.29 (60.84)	76.66 (61.09)
Buds inoculation with hypodermic syringe	62.22 (52.07)	62.70 (52.93)	62.46 (52.50)	63.70 (53.02)	65.92 (54.27)	64.81 (53.64)
Buds wrapping by cotton swab dipped in smut suspension	71.84 (57.97)	71.84 (57.95)	71.84 (57.96)	72.58 (58.43)	71.84 (58.00)	72.21 (58.21)
Inoculation of underground buds at time of tillering stage	71.10 (57.47)	74.81 (59.88)	72.97 (58.67)	73.33 (58.89)	73.32 (58.93)	73.33 (58.91)
Mean	70.36 (57.09)	71.78 (58.16)		71.66 (57.92)	71.84 (58.01)	
CD (p=0.05) level for						
Inoculation technique		2.08			3.57	
Types of spores (Teliospores and sporidia)		NS			NS	
Inoculation techniques x types of spores		NS			NS	

Figure within parentheses represent arc sine transformed values and CD is applicable to these only. *Average sett germination of three replications.

Table 2. Effect of different smut inoculations techniques on smut incidence.

Inoculation technique	Percent smut incidence		
	Teliospores	Sporidia	Mean
Inoculation of setts by dipping in spore suspension	54.84* (47.76)	33.07 (35.02)	43.95 (41.39)
Buds inoculation with hypodermic syringe	69.78 (56.64)	51.48 (45.84)	60.63 (51.24)
Buds wrapping by cotton swab dipped in smut suspension	55.02 (47.88)	34.15 (35.73)	44.58 (41.80)
Inoculation of underground buds at time of tillering stage	12.12 (20.36)	4.99 (12.79)	8.55 (16.58)
Mean	47.94 (43.16)	31.17 (32.34)	

CD (p=0.05) level for: Inoculation technique = 3.20; Types of spores (teliospores and sporidia) = 2.26; Inoculation techniques x types of spores = NS; *Average disease incidence from June to 15th February 2007. Figure within parentheses represents arc sine transformed values and CD is applicable to these only.

and 45 days of sowing respectively, in bud inoculated with hypodermic syringe. No significant difference was observed when the sets were dipped in teliospores or sporidia. Similarly no significant correlation was observed between inoculation techniques and types of spores.

The maximum disease incidence (60.63%) was observed when the buds were inoculated with

hypodermic syringe and minimum incidence (8.55%) was recorded in inoculation of underground bud at the time of tillering stage (Table 2). The disease incidence of 44.58% was recorded when the buds were wrapped by cotton swab dipped in smut spore suspension and 43.95% in inoculation of sets by dipping in spore suspension.

Significant difference was observed in smut incidence (%) when the buds was inoculated by hypodermic syringe and inoculation of underground bud at time of tillering. Significant difference in smut incidence was also observed when the inoculation was carried by teliospores and sporidia separately. The mean disease incidence was 47.94% when sets were inoculated

with teliospores and it was only 31.17% by sporidial inoculation (Table 2). Inoculation is through mechanical injury, that is, hypodermic syringe, increase smut disease incidence and decrease in the germination as compared to inoculation without injury. Waller (1970) made a pioneering work in comparing different methods of smut inoculation and found that injection inoculation may induce greater smut infection than dip inoculation which is confirmation of our study. Olweny et al. (2008) critically evaluated the smut inoculation techniques in sugarcane seedlings and explored the possibility of screening for smut resistance at the seedling stage. Dalvi et al. (2011) also used artificial inoculation of smut sori to sugarcane sets by dipping into smut spore suspension for 30 min and planted in field for screening of somaclones and achieved significant results. The findings of other workers (Luthra et al., 1938; Waraitch and Kumar, 1987; Duttamajumder, 2000) are also in accordance with our study.

Duttamajumder (2000) reported that inoculation of underground buds at the time of tillering gave 83% disease incidence as compared to 36% in dipping of setts in spore suspension but the present study negates their observations.

Laboratory evaluation

In tissue culture raised plantlets, no smut sori were developed. However, mycelium of the fungus was present in the leaves, which is not a true indication of disease development in this experiment. Therefore, this method is not to be considered as a substitute for field screening which is more effective. Singh et al. (2005) tried smut screening on tissue cultured sugarcane plantlets and reported that the method is not a substitute for field screening. The present study also did not give any positive direction towards the quick screening technique.

Conflict of Interests

The authors have not declared any conflict of interests.

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