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Evaluation of Smut Inoculation Techniques in Screening of Varieties for Sugarcane Smut (*Sporisorium scitamineum*) Resistance at Ethiopian Sugar Estates

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Abstract

Field experiment was conducted at Wonji shoa sugar estate, to evaluate the effectiveness of different smut inoculation techniques on expressing the smut disease symptom and its effect on sugarcane bud sprout and tiller population. Three sugarcane smut inoculation techniques (dip inoculation, wound paste and paste without wound) were evaluated using three sugarcane varieties having known smut reaction group. The treatment combinations were arranged in factorial Randomized Complete Block Design (RCBD) with three replications. Data on number of smut whips, incidence, sprout and tillers count was recorded. Individual analysis of variance was performed using the SAS Statistical Software package. Analysis of variance for the field experiment indicated that there was no interaction between variety and inoculation techniques for all dependent variables. However, both smut inoculation techniques and varieties were significantly affect the smut incidence and sprout while there effect on tiller population was non-significant. On average the wound paste method was found to be superior on whip production and percent infected stools (21.37) over the dip and paste without wound methods of inoculation. On the other hand, the dip and paste without wound technique of inoculation showed non-significant difference for their mean percent smut infected stools while they remain significant on whip production per hectare. Among the sugarcane varieties evaluated, smut resistant variety showed statistically significant difference for mean percent smut infected stools. Among the smut inoculation techniques were under the test, on average plot received wound paste showed the lowest (44.77%) percent sprout as compared to paste without wound and dip techniques of inoculation. Since the wound paste techniques of inoculation outsmart in mean percent smut incidence and whip production over dip and paste methods, it was recommended to be used for screening of sugarcane variety/s for smut resistance.

Keywords: Inoculation; Resistance whips; Inoculation; Sugarcane

Introduction

Due to its vegetative mode of propagation, sugarcane (*Saccharum* spp.) is prone to infection by systemic pathogens. The most effective and cheapest method of controlling sugarcane diseases is the use of resistant cultivars [1]. The most widely used technique to evaluate for resistance to smut involves immersing sugarcane setts (seed pieces with 1-3 nodal buds) in a recommended rate (5 $ml^{-1} \times 10^6$ ml^{-1}) of teliospores suspension that initiate the natural infection rate of the pathogen before planting and counting the number of sori (whips) that develop [2]. To assess smut reaction, typically a dip inoculation assay is used in which nodal buds are immersed briefly in a suspension of teliospores and then planted in a greenhouse. However, evaluation can also be takes place in a greenhouse or in the field [3].

Report show that when available resistant is short lived, it could be attributed to pathogen genetic variability. A recent survey carried out in western Kenya showed that some varieties susceptible to smut in one location were immune or resistant in other locations indicating genetic diversity of smut pathogen races. Moreover, the sugarcane varieties in Kenya are polyploidy of several *Saccharum* species where by genetic resistance for smut do not follow gene for pattern. Varietal differences in susceptibility to different smut isolate have been reported [4-6]. Smut rating and ranking of the cultivars can vary significantly from year to year since host reaction to smut is dependent on the environment and probably race of the pathogen present [7]. Because host resistance is the most cost effective and easy control method, many resistant varieties have been developed.

Consequently for the management of sugarcane smut, the Ethiopian sugarcane plantations have been practicing cultural, mechanical heat water treatment and host plant resistance management system. However on average more than two million affected stools are rouged per year with an operation cost of about ten million Ethiopian birr every year. As a result, some high yielding sugarcane cultivars (like Co 419, Co 421, NCo 310, NCo 376 and M165/38) have been put out of production due to their high susceptibility to the disease in the sugarcane plantation of Ethiopia [8].

To identify resistant clones, it is necessary to develop inoculation techniques to ascertain their disease reaction. Generally, two methods have been used: Field infection and artificial inoculations. The first

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method gives an excellent evaluation of how a clone will react in the field, but it has limitations. Large areas are required, a naturally high inoculums density must be present and a relatively long period is required to obtain results. When thousands of seedlings need to be tested, the logistics of such testing are prohibitive. Artificial inoculation methods also have advantages and disadvantages.

Large numbers of clones can be handled, but interpretation of the results and how they relate to natural field conditions can be a problem. Since we had anticipated testing large numbers of seedlings, the artificial inoculation method seemed more desirable.

Basically, three methods have been utilized by others to inoculate setts; dipping in or spraying on a spore solution; or painting a spore paste over buds which have been wounded by needle punctures [9].

The current resistance screening method for sugarcane smut involves dipping setts into a smut spore suspension and inspecting for smut whips in a short plant and ratoon crop cycle. The method is very effective for screening a large number of varieties in a relatively short period of time. Although this method is internationally accepted, it has some drawbacks:

- It does not replicate natural infection.
- Plants are subject to very high disease pressure.

Even though, no physiological race difference of sugarcane smut was existed according to in Ethiopian sugar estate, beside environmental factors, the cause why the same variety responded differently across location might be attributed to environmental by Gene Interaction (GBI) and the effectiveness of smut inoculation method have been used before to screen varieties for smut resistant is inconsistent [10,11]. As the result, the sugarcane varieties such as B41/227, N52/219 and N53/216 which previously categorized as smut resistance according to the report indicated on review of sugarcane research in Ethiopia II. Crop protection from 1970-1998 were highly infected currently at Wonji Shoa and Kuraz (N52/219 and N53/216) and Tendaho (B41/227) sugar estates.

In addition, in sugarcane little work has been done on screening of varieties with smut and evaluating the inoculation methods other than dip inoculation in the Ethiopian sugar estates.

Therefore, the present study was initiated with the following objectives:

- To evaluate the effectiveness of different inoculation techniques on expressing the disease symptom.
- To evaluate the effect of different inoculation techniques on sugarcane bud sprout and tiller population.

Materials and Methods

Description of the study area

Field experiment to evaluate smut inoculation techniques was conducted at Wonji Shoa sugar estate, Ethiopia, in 2014/15 cropping season. The site is located in the central east part of the main Ethiopian rift valley system, Adama Woreda, Eastern shoa zone, Oromia national regional state, 110 km from South East of Addis Ababa and about 10 km South of Adama town. It is found between 8°31' N and 39°12' E and at an altitude of 1550 m.a.s.l. Average maximum and minimum temperatures is 27.6°C and 15.3°C, respectively. It receives an average annual rainfall of 813 mm and peak daily evapo transpiration of 4.5 mm [12].

Sugarcane smut disease inoculation types

Sugarcane setts were inoculated with smut spores suspension in three different ways.

- The setts were soaked in a smut spore suspension (5 grammas of spores per 1 liter of sterile water at 5 × 10 spores/ ml) for 30 minutes.
- The setts were wounded at the bud with a scalpel then a paste of smut spores suspension made at a concentration of 2 grammas of spore for 2 ml of sterile water was applied.
- A paste of smut spore suspension was painted at the buds of the setts.

Field study approach

The study was conducted at Wonji plantation fields as of 2015 for one cropping season. In this experiment, three sugarcane smut inoculation techniques (dip inoculation, wound paste and paste without wound) were evaluated using three sugarcane varieties having different known smut tolerance level (resistance-52/298, moderately susceptible N-14 and susceptible–Nco 310) against smut disease (Table 1).

The treatment combinations were arranged in factorial Randomized Complete Block Design (RCBD) with three replications. A plot size of six furrows of seven meter length (60.9 m^2) was used and 40 double budded setts were planted per furrow at the depth of 2.5 cm for each inoculation method. Data collection and observation were made on the middle four furrows of each plot. All the recommended cultural practices of the estate except, smut control measures were applied as per the estate.

Sugarcane smut disease inoculation techniques

In this experiment, ten to twelve month old double budded sets of each variety (resistance, moderately susceptible and susceptible) were used as a planting material from the nursery field of the plantation. Smut resistance (B52/298) and moderately susceptible (N 14) varieties used in the experiment were received hot water treatment at 50°C for 2 hrs. to remove any sett borne diseases while smut susceptible variety NCo-310 sourced from tissue culture seed cane fields. The leaves along with the sheaths of the stalks were detached to expose the buds and cut into double budded setts. To swell up the buds and ensure their susceptibility, the setts were incubated for overnight in a polythene bag filled with a liter of water following procedure of sugarcane smuts spores were collected from Wonji Shoa infected sugarcane fields and were tested for viability by transferring the spores into a glass slide with a drop of distilled water using needle, which is placed onto a petri plate that has a filter paper moistened with distilled water.

The petri plate that contained the spore suspension was incubated for 8 hrs and the germinated spores were counted by mounting the glass slide under the microscope. The concentrations of teliospores were standardized by using haemocytometer. A spore of 0.1 g were measured and added to 75 ml of water. Tween 20 was added to homogenize the spore suspension. Then, some drops of the suspension were placed on the engraved grid of the haemocytometer and put on the cover glass of the haemocytometer over the grid. The average numbers of spores were calculated from three counts taken from five points of the middle square of the haemocytometer. The obtained number of spores were multiplied by 25 which is that the middle square has 25 smaller squares and because the average number of

spores represented only one of the smaller squares. The resulting number was multiplied by the volume (10^4) of the bigger square.

The final results of the spore counts were adjusted until the standard spore concentration (5×10^6 ml^{-1}) was obtained which required for initiating the natural infection rate of the pathogen. The incubated, setts were used for each inoculation technique with the respective spore concentration. To create favorable environmental conditions for disease development, the inoculated setts were incubated for overnight in polythene bag filled with a liter of water just after inoculation.

SN	Treatments
T1	B52/298+Dip inoculation
T2	B52/298+Wound paste
T3	B52/298+Paste without wound
T4	N14+Dip inoculation
T5	N14+Wound paste
T6	N14+Paste without wound
T7	NCo-310+Dip inoculation
T8	NCo-310+Wound paste
T9	NCo-310 +Paste without wound
NB: Where, B 52/298=Resistant variety, N14=Moderately susceptible and NCo-310=Susceptible variety	

Table 1: Treatment combinations.

Data collection

Data on number of smut whips per stool, smut incidence per population sprout count and number of tillers population per plot were recorded. Data collection on sugarcane varieties to smut infection, were began two months after inoculation. Data on sprout count and tiller population were taken at 45 days and four month after planting respectively. Except sprout and tiller count, the data collection were continued for eight months, at one month, 15 and 10 days intervals for resistant, moderately susceptible and susceptible varieties respectively. The incidence of the disease was computed using Seem formula as indicated below.

$$\text{Smut incidence (\%)} = \frac{Total\ number\ of\ infected\ stools/plot}{Total\ number\ of\ stools/plot} \times 100$$

Data analysis

Individual analysis of variance for the field experiment, were performed using the SAS statistical software package on plot means of tiller population and disease incidence. The effect of inoculation on the sugarcane setts and the interactions were evaluated. Mean separation and comparisons were tested by List Significant Difference (LSD).

Results and Discussion

The interaction effect between the smut inoculation techniques and the sugarcane varieties

There was no interaction between the two main factors for all dependent variables such as incidence, sprout and tiller population (Table 2). However, smut inoculation techniques significantly affect the smut incidence and sprout while its effect on tiller population was non-significant. Similarly, variety was also affects the smut incidence and sprout significantly at 5% probability level, while it remains non-significant on tiller population. Since, there was no interaction effect between the two main factors variety and inoculation techniques, only the main effect were compared based on their mean comparison.

		Incidence (%)	Sprout (%)	Tiller ('000 ha^{-1})
1	Technique	***	***	ns
2	Variety	***	***	ns
3	Technique *variety	ns	ns	ns
Mean		15.15	56.26	4042
CV (%)		18.1	11.25	27.9
LSD		5.474	13.21	186.87
*NB: Where, *Stands for significance and ns: non-significance				

Table 2: ANOVA table for dependent variable.

Effect of smut inoculation techniques on sugarcane smut disease expression

The study revealed that there was statistically significant difference observed between the smut inoculation techniques at 5% level of probability for whip production. Accordingly, on average the wound paste method was found to be superior on whip production with a value of 4914 per hectare which is followed by the paste without wound technique with 4447 whips/ha of inoculation (Table 3). Similarly, Baythe and Steiner compare the dip, spray, wound paste and paste methods of inoculation on sugarcane seedlings using 10 clones whose field reaction to smut was known and he reported that the wound paste method achieved a higher degree of infection. On the other hand, Baythe and Steiner also reported that, the wound paste technique seems to be a strict test, but it may have usefulness in a program aimed at selecting clones with a high degree of resistance [13].

Inoculation techniques	% smut affected sools/plot	Number of smut whips/ha
Dip	10.48^{b}	3175^{c}
Wound paste	21.37^{a}	4914^{a}
Paste without wound	13.60^{b}	4447^{b}
CV%	18.1	18.09
LSD	5.474	327
NB: Means followed by the same letter along columns are statistically non-significant at 5% probability level according to LSD test		

Table 3: Effect of smut inoculation techniques on sugarcane smut disease expression.

Moreover, among the smut inoculation techniques evaluated, the wound paste method scores the highest percent infected stools over the dip and paste without wound methods of inoculation. This clearly indicates that wound paste technique of inoculation was a promising for screening of sugarcane varieties for smut resistance. In addition, this effective inoculation technique had a significant effect on screening for smut resistance at the first stage of selection and to avoid carrying large numbers of clones that will eventually be discarded at the advanced stage of selection and it also requires less amount of teliospores concentration as compared to the dip inoculation techniques for the initiation of natural infection rate of the pathogen. In similar manner Ragaand Bukhari tested the pin prick, dip and natural spreader row infection method of artificial inoculation and the mean percentage of smut infection was 2.81, 1.96 and 2.26 for pin prick, dip and natural spreader row infection in Plant Cane (PC); 5.51,4.39 and 7.4 for the first Ratoon (Rl) and 6.54, 6.06, 8.66 for the second Ratoon crop (R2), respectively and the pin prick and natural spreader row infection gave slightly high mean percentage of infection values in PC and R1 crop [14]. On the other hand, the dip and paste without wound technique of inoculation showed non-significant difference for their mean percent smut infected stools. However, these two treatments showed significant difference on whip production per hectare.

Effect of smut incidence on sugarcane verities

Smut resistant variety (B 52/298) showed statistically significant difference for its mean percent smut infected stools as compared to smut susceptible (NCo 310) and moderately susceptible (N 14) varieties (Table 4). However, among the sugarcane varieties evaluated, the smut susceptible and moderately susceptible variety were showed statistically a non-significant difference in their disease expression potential.

Verities	% smut affected sools/plot
B52/298	3.38^{b}
N14	17.73^{a}
NCo-310	19.34^{a}
CV%	19.4
LSD	3.52
NB: Means followed by the same letter along columns are statistically non-significant at 5% probability level according to Lsd test	

Table 4: Effect of smut incidence on sugarcane verities.

This might be attributed to the genetic makeup of variety (B52/298) which is responsible for smut disease resistance resulted, a significant variation in expression of the smut disease symptom as compared to he smut susceptible and moderately susceptible varieties. In addition, among the factors affecting the occurrence of infection by specific pathogen is governed by the susceptible

gene present in the host plant. Therefore, even though this variety was exposed to different techniques of inoculation, difference in disease expression potential on resistant variety was not expected whatever the technique of inoculation is.

Except the smut resistant variety B52/298, the remaining two sugarcane varieties (NCo-310 and N14) which belongs to a known smut reaction group of the susceptible and moderately susceptible were respond non-significantly for percent smut infected stools. The non-variation between smut susceptible and moderately susceptible verities was come from the cumulative effect of the three technique of inoculation were the varieties subjected minimized the variation in disease expression potential.

Effect of smut inoculation techniques on sugarcane bud sprouting

Wound paste inoculation technique was significantly affecting sugarcane bud sprouting. Accordingly, among the smut inoculation techniques evaluated, on average plot received wound paste showed the lowest (44.77%) percent sprout as compared to paste without wound and dip techniques of inoculation. As the result of inoculation had a significant effect on sugarcane bud sprouting, the lowest sprout percentage on wound paste as compared to the remaining techniques of inoculation might be come up from the disturbance or mechanical damage of sugarcane bud by scalpel to create a wound during inoculation processes.

Similarly, Paramdeep, et al. reported that, inoculation through mechanical injury significantly increase disease incidence but, at the same time also affect the bud germination (15.56%). On the other hand relatively the highest (64.28%) mean percent sprout was recorded on plots received paste without wound technique of inoculation (Table 5). Therefore, the overall percent germination falls in a range of the standard percent germination of Wonji Shoa sugar estate.

Inoculation techniques	Sprout (%)	Tiller ('000/ha)
Dip	59.73[a]	183.22[a]
Wound paste	44.77[b]	137.11[b]
Paste without wound	64.28[a]	197.00[a]
CV%	11.25	11.57
LSD	13.21	39.862
NB: Means followed by the same letter along columns are statistically non-significant at 5% probability level according to Lsd test.		

Table 5: Effect of smut inoculation techniques on sugarcane bud sprout and tiller population.

Effect of smut inoculation techniques on sugarcane tiller population

Among the smut inoculation techniques were under the test, the wound paste showed statistically significant difference for sugarcane tiller population. However, the dip and paste without wound techniques of inoculation remains non-significant from each other. As the result, the tillering rates, the rate at which young shoots appear were decreases on plot received wound paste technique of inoculation. Therefore, the lowest (137.11,000/ha) tiller population on wound paste inoculation technique might be come from the lowest sprout percent resulted from the mechanical damage of the sugarcane bud by scalpel to create wound during inoculation process. In line with the above result, Waller reported that the tillering rate has been reported to progressively decrease in the field infected sugarcane cultivars [15]. On the other hand, the non-significant variation in tiller population between dip and paste without wound techniques of inoculation might be attributed to in both inoculation techniques there was no mechanical bud damage in the inoculation process.

Economic benefit

Knowing of the response of specific variety to major economic disease is an important factor to set effective management strategy. So, sugarcane smut is one of the major diseases in the Ethiopian sugar industry which causing 19%-43% and 30%-43% cane yield and sugar yield loss respectively. In addition, on average more than two million affected stools are rouged per year with an operation cost of about ten million Ethiopian Birr every year. As a result, some sugarcane cultivars (like Co 419, Co 421, NCo 310, NCo 376 and M165/38) have been put out of production due to their high susceptibility to the disease in the sugarcane plantation of Ethiopia [16-18]. Therefore, in line with the above facts, the present finding was minimize the yield loss and rouging operational cost indicated above.

Conclusion

Effective inoculation technique had a significant effect on screening for smut resistance at the first stage of selection and to avoid carrying large numbers of clones that will eventually be discarded at the advanced stage of selection. The wound paste techniques of inoculation outsmart in mean percent smut incidence and whip production over dip and paste methods.

Although, the wound paste technique of inoculation adversely affect the sugarcane bud sprout, as compared to dip and paste without wound inoculation techniques, it's not run out of the average standard

percent sprout of Wonji-Shoa sugar estate and also not maintain its adverse effect on tiller population except for the plots received wound paste inoculation technique. Therefore, its effect on yield would be also minimal. Therefore, based on the present finding, the following recommendation was made.

Recommendations

The wound paste method of inoculation was recommended to be used by research and development center for screening of sugarcane variety/s for smut (*Sporosorium scitaminea*) resistance, because of the following reasons:

- It was found a promising for screening of sugarcane varieties for smut resistance since it resulted the highest percent smut incidence.
- It needs relatively small amount of teliospores concentration than dip method of inoculation to initiate the natural infection rate of the pathogen.

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