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Ethiopian Sugar Landraces Screening Against Sugar Cane Smut (*Sporisorium scitamineum*) at Metahara Sugar Plantation

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Abstract

Background and Objective: Sugar cane smut is one of the most yield-reducing factors and is widely spreading and established elsewhere where, sugar cane is prevalent. The use of resistant varieties is among the most economical and environmentally the safest way to manage sugar cane smut. Hence, twenty Ethiopian landrace sugar accessions along with two standard checks were screened for their resistance against sugar cane smut disease at Metahara Sugar Estate to identify the resistant sugar cane accessions with better agronomic performance and resistant yielder under the existing field condition of Metahara plantation. **Materials and Methods:** The experiment was executed in the 2020 cropping year. A single bud setts were used as planting materials and then, inoculated by soaking method into smut spore suspension that was made at a concentration of 5×10^6 teliospores/mL for 30 min. **Results:** The results of this screening activity revealed that several promising resistant varieties were identified from the Ethiopian landraces. Based on the current screening activities, about 90% (18 out of 20) of local landraces have shown high resistance to a very highly resistant reaction while 10% (2 out of 20) of the candidates showed a resistant reaction during the life span of evaluation time. From the current smut screening activity, a reaction range of resistant to very highly resistant was identified. **Conclusion:** Results concluded that almost all the Ethiopian landraces were promoted for the next breeding evaluation steps. The use of resistant sugar cane varieties is among the method used to reduce the rate of sugar cane expansion in commercial plantations.

Key words: Landraces, screening, smut, accessions, sugar cane, inoculation, single bud sett

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sugarcane smut is now seen as being widely spread and established everywhere sugar cane production is prevalent¹. Sugar cane smut has a systemic mode of infection and as a consequence, it reduces crop growth, stalk diameter, yields and industry profitability. Sugar cane smut could result in a terrible yield loss. Stunted cane is a manifestation of severe infestation by sugar cane smut diseases^{2,3}. Research findings by Mansoor *et al.*⁴ revealed that sugar cane smut contributed to a quantitative and qualitative loss in susceptible varieties. Magarey *et al.*⁵ reported, that the yield loss due to smut could reach 62% to a total crop failure when susceptible cultivars are planted. In the Ethiopian sugar estates a loss of 19-43% in cane and 29.5-42.8% in sugar yield.

The currently growing sugar cane varieties are very susceptible. Even though the so far resistant varieties are becoming susceptible. Most of the cultivars that are cultivated in Ethiopian sugar plantation were introduced from abroad where diversity is available with giving no or less attention to the native sugar cane varieties.

Therefore, to affect this collection of diverse germplasm sources from the different agroecological zone is highly essential to get sufficient newer resistance accessions to sugar cane smut disease. Hence, these tested materials were collected from almost all corners of Ethiopia to increase the possibility of getting resistant sugar cane varieties to slow down the ever-increasing of sugar cane smut disease in many Ethiopian sugar plantations. An important strategy to control smut disease on a broad scale is achieved through the selection and planting of resistant sugarcane cultivars. Genetically resistant variety/cultivar is one of the most effective and environmentally friendly and socially acceptable methods of disease control according to a report by Gray *et al.*⁶.

The aspect of varietal evaluation for the disease reaction has been adopted by introducing sugar cane varieties from elsewhere where diverse sugarcane accessions are available reported by Dalvi *et al.*⁷. Identification of resistant cultivars needs elite sources of resistance to smut. A high-yielder and resistant varieties require a continuous way to supply new germplasms as a source of desirable genes and/or gene of complexes and the primary sources of such genes are landraces, introductions, weedy and wild relatives of crop plants as reported by Gashaw *et al.*⁸.

Therefore, to affect the successful varieties screening for resistance against sugar cane smut it requires (i) The availability of large and diverse germplasm collections, including wild species, (ii) The knowledge of both plant and

pathogen biology (iii) The availability of precise and accurate screening techniques. According, to Rutkoski *et al.*⁹ smut-resistant lines were the prime focus for being used in cane breeding programs as these lines don't allow the pathogen to cause disease. As suggested by Brown¹⁰ the merit associated with Landraces is widely adapted to specific agro-climatic conditions while maintaining considerable diversity between and within populations, constituting a reservoir of genetic diversity that is interesting for future breeding work as well for the development of new agricultural systems and new products.

Therefore, the exploration of their genetic diversity and conservation for future generations is important. Currently, occasional infections have been reported from resistance variety by *S. scitamineum* reported this probably happened due to the deterioration or variation in the pathogen population Dalvi *et al.*⁷. To assess such a reaction, researchers typically use the soaking inoculation method as described by Shen *et al.*¹¹. Resistance to sugar cane plays an immense role in smut disease management.

This research activity was initiated to screen the introduced and Ethiopian landraces for their reaction against sugar cane smut and to promote resistant accessions for their further breeding and agronomic evaluations.

MATERIALS AND METHODS

Study area: Metahara Sugar estate is located in the Oromia Region about 200 km southeast of the Capital City, Addis Ababa. It is situated at 8°53'N and 39°52'E with an altitude of 950 m above sea level (m.a.s.l). The experiment was conducted during the cropping year of 2019/2020.

Treatments and experimental design: Twenty landraces accessions were evaluated along with two standard checks (C86/56 and NCo334), i.e., highly resistant and highly susceptible, respectively. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. A plot size of 4 furrows (1.45 m×4) by 5 m was used. A space of 3×2 m between block and plot was used, respectively. Sugarcane smut spores were collected from commercial fields of Metehara, following the procedures of Tokeshi. A 540 single bud was used as planting materials for each accession. The prepared single bud setts were incubated under humid conditions in polythene bags for 24 hrs before inoculation to create a favourable condition for infection. Then, soaking was done into smut spore suspension made using 1 g of teliospores and in 1 L of water. The soaking time was 30 min. Before inoculation activity was performed

teliospores viability test was conducted. Then, the hemocytometer was used for the concentration adjustment to 5×10^6 teliospores/mL. This was the concentration required to initiate smut disease development under natural conditions.

Smut disease incidence: Incidence was computed using the following formula described by Chiang *et al.*¹²:

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

The number of infected stalks/ha was also calculated from the number of smut-affected tillers, which by itself was calculated from the number of smut-affected stools of the field as described by Bhuiyan *et al.*¹³.

The total number of smut-affected tillers (Ts) was calculated as:

$$Ts = Ss \times 7$$

where, Ss is the total number of smut-affected stools and 7 is the average number of tillers produced per stool. From the total smut-affected tillers data, a total number of smut-affected stalks (STs) was calculated as:

$$STs = \frac{Ts \times 56}{100}$$

where, Ts is the total number of smut-affected tillers and 56 is the percentage of tillers that reach the millable stalk.

Stalk loss in quintals (Qt ha⁻¹): Stalk loss in quintals was calculated as the number of smut-infected stalks*weight of smut-free stalks.

Resistance assessment: A numerical rating scale of 1-9 where, 1 = Highly resistant and 9 = Highly susceptible was used as described by Lemma *et al.*¹⁴ for landraces reaction assessment.

Data analysis: Data on cane percentage of smutted stools incidence, number of the smutted stalk, the total number of smut affected tiller severity and stalk loss in ton/ha were subjected to analysis of variance by using the methods described by Gomez and Gomez (1984) using SAS computer software. Mean separation was based on LSD at a 5% level probability level.

RESULTS AND DISCUSSION

Number of smutted stools (count/ha): The highest number of 7084 smutted stools/ha and statistically significantly different from the other candidates was recorded from the NCo334 (susceptible standard), but, statistically at par with local land-races, 159, 159, 159, 159, 159, 153, 141, 422 and 188. The least smutted stool count or zero was recorded from the resistant standard check (Table 1). Among the evaluated land races five sugar cane accessions namely, 43, 164, 189, 43, 164, 189, 190 and 203 showed a statistically non-significant difference from the resistant standard check. Due to the dismal cane harvesting operation in Ethiopian sugar plantations, sugar cane variety with some number of smutted stools are no further free from sugar cane smut disease slays as the rationing cycles increase. Rouging out of diseased stools is not recommended except where whip counts are below 5% (600 stools per hectare) or in small fields or nurseries. Ten local land-races accessions (7, 139, 157, Mori 60, 140, 138, 139, 157, 177 and 43) showed statistically significant ($p < 5\%$) differences from the susceptible and resistant standard check, which account for 50% of the local land-races accessions. Cultivation of resistant accessions is necessary for avoiding the sugar cane smut disease. Research findings by Croft *et al.*¹⁵ revealed that complete eradication of sugar cane smut is not possible as it is a soil-borne disease and it may spread out in the entire area under cane cultivation. The use of smut-resistant lines was the only viable option to slow down the rate epidemic in commercial sugar plantations.

Percentage of smutted stools incidence (%) and smutted stalks incidence (%) of Ethiopian landraces: The highest 27.664% and statistically significant difference from all the Ethiopian landraces were recorded from the susceptible standard check. The lowest smut incidence (0%) was recorded from the resistant standard check.

Among the local land-races accessions, accession 4, 7, 46, 138, 139, 141, 153, 164, 188, 189, 190 and 203 showed statistically analogous to resistant standard check (C86/56), but, statistically significant from land-races 159, 151 and 43. These accessions account for 65% of the total evaluated Ethiopian landraces. On the other hand, accessions 43, 140, 151, 157, 159, 177 and 422 showed statistically significantly different from the standard resistant checks ($p < 0.001$) these account for 35% of the total evaluated candidates. Similarly, accessions 43, 151 and 159 showed statistically significantly

Table 1: Mean number of sprouts count, smutted stools, smutted infected stalk, smutted stools incidence, smutted stalk incidence, the weight of smut-free stalk(kg/stalk) and stalk loss (Qt ha⁻¹) for Ethiopian landraces

Accessions	Number of sprouts or stools/ha	Number of smutted stools/ha	Number of smutted stalks/ha	Smutted incidence (%)	Smutted stalk incidence (%)	Weight of smut-free stalk (kg/stalk)	Stalk loss in Qt ha ⁻¹
Mori 60	27127.59 ^{ab}	252.56 ^{cd}	2344 ^{ih}	0.931 ^{fg}	1.116 ^{fg}	2.49 ^a	58.34 ^e
159	21610.34 ^{b-f}	1836.88 ^b	21003 ^{ab}	8.5 ^b	4.276 ^{b-f}	1.35 ^{ef}	283.54 ^{a-d}
140	25172.41 ^{a-d}	1429.04 ^b	14340 ^{b-g}	5.677 ^{b-f}	3.188 ^{c-g}	2.44 ^a	349.86 ^{abc}
151	17586.20 ^{e-h}	1385.44 ^b	7137 ^{c-h}	7.878 ^{bc}	4.606 ^{b-e}	1.40 ^{ef}	99.918 ^{ed}
190	30113.79 ^a	1392.16 ^b	11051 ^{b-h}	4.623 ^{b-g}	1.839 ^{d-g}	1.17 ^f	129.29 ^{c-e}
153	20458.62 ^{b-f}	970.76 ^{bc}	11675 ^{b-h}	4.745 ^{b-g}	4.702 ^{b-d}	1.52 ^{c-f}	177.46 ^{c-e}
141	26896.55 ^{a-c}	986.03 ^{a-c}	9333 ^{ef}	3.666 ^{b-g}	3.861 ^{b-f}	2.19 ^{ab}	204.39 ^{b-e}
422	22758.62 ^{b-e}	1440.62 ^b	16040 ^{b-e}	6.330 ^{b-d}	6.395 ^{bc}	1.86 ^{a-e}	291.8 ^{a-d}
203	19424.13 ^{d-f}	256.59 ^{cd}	3338 ^{ihg}	1.321 ^{e-g}	3.076 ^{c-g}	1.11 ^f	37.05 ^e
188	15172.41 ^{f-h}	385.68 ^{cdg}	7339 ^{c-h}	2.542 ^{d-g}	6.825 ^b	1.34 ^{ef}	98.34 ^{de}
46	25172.41 ^{a-d}	565.12 ^{cdg}	6002 ^{d-h}	2.245 ^{d-g}	1.981 ^{d-g}	2.27 ^a	136.24 ^{c-e}
43	23562.06 ^{a-e}	1785.06 ^b	17333 ^{bc}	7.576 ^{abc}	2.5 ^{d-g}	1.88 ^{a-e}	333.9 ^{abc}
7	14596.55 ^{f-h}	296.46 ^{cdg}	4004 ^{fg}	2.031 ^{d-g}	4.473 ^{b-f}	2.38 ^a	83.7 ^{de}
189	24827.58 ^{a-e}	427.03 ^{cdg}	4370 ^{fg}	1.720 ^{e-g}	1.890 ^{d-g}	2.36 ^a	98.2 ^{de}
139	20458.62 ^{b-f}	265.35 ^{de}	2686 ^{f-h}	1.297 ^{e-g}	1.122 ^{fg}	2.22 ^a	65.2 ^e
138	23448.27 ^{a-e}	481.63 ^{ce}	5071 ^{e-h}	2.054 ^{d-g}	3.367 ^{c-g}	2.16 ^{a-c}	109.9 ^{de}
164	20000.0 ^{c-f}	620.40 ^{cf}	8004 ^{c-h}	3.102 ^{c-g}	2.866 ^{d-g}	1.51 ^{d-f}	119.6 ^{de}
4	12644.82 ^{gh}	86.62 ^{dg}	2000 ^{gh}	0.685 ^g	1.190 ^{e-g}	2.10 ^{a-d}	45.0 ^e
157	19310.34 ^{e-g}	1104.74 ^b	15039 ^{b-f}	5.721 ^{b-f}	4.354 ^{b-f}	2.34 ^a	357.9 ^{ab}
177	20920.68 ^{b-f}	1239.76 ^b	16192 ^{b-d}	5.926 ^{b-e}	3.367 ^{b-g}	2.16 ^{a-c}	340.1 ^{ab}
NCO-334	25610.344 ^{a-d}	7084.0 ^a	30409 ^a	17.664 ^a	27.664 ^a	1.48 ^{e-f}	449.2 ^a
C86-56	22527.58 ^{b-e}	0.00 ^f	0 ^h	00.0 ^a	00.00 ^a	2.13 ^{ab}	0.0 ^e
Rsq	66.9	62.78	65.59	61.0	75.52	67.0	56.0
CV	27.67	34.07	36.33	28.0	35.24	20.70	37.56
LSD	6896.55	614.48	11076	4.98	3.44	0.641	219.77

Mean values followed by similar letters within the column and with the same factor are not significant at the 5% level of probability use and ns: Non-significant

different from all the other landraces in their percentages of smutted stools/ha incidence which are very close to moderately resistant. Therefore, in this work, several elite sugar cane accessions were identified from the Ethiopian landraces, which provides an immense opportunity for the Ethiopian sugar industry to replace the inferior imported materials that were introduced from abroad. Ethiopian landraces consist of several important genotypes that may be used for sugar breeding owing because of their potential adaption to their specific environmental condition and their large genetic variability among them. This variability is enhanced when the collection is done over a wide range of environments. However, according to Gepts¹⁶. The highest number of smutted stalks incidence of (11%) was recorded in NCo334 followed by sugar cane accessions number 159 (8.5%) and accession number 151 (7.87%) while, the least smut stalk incidence of zero was recorded from C86/56 which was statistically at par with 4, 7, 46, 138, 139, 141, 153, 164, 189, 190 and 203. Land-races accessions response to sugar cane smut disease. Results of land-races screening have shown that out of 20 land-race accessions, nine (9) accessions were found as very highly resistant (4, 7, 46, Mori 60, 138, 139, 188, 189 and 203), four accessions (141, 153, 164 and 190) highly resistant, five accessions (140, 151, 157, 177 and 422) resistant and two (43 and 159) moderately resistant (Table 2). Ethiopian

land-races accessions showed a smut stools percentage (%) value ranging from 0.68-8.5% (Table 2). Amongst, the evaluated landrace, none of them showed an immune reaction and a response less than moderate resistant reactions. About 65% (13 accessions out of 20) of landraces showed a response reaction ranging from a highly resistant to a very highly resistant reaction while 35% (7 accessions out of 20) candidates' showed resistance to moderate resistance against smut disease during the life span of the evaluation time. Therefore, current findings revealed that Ethiopian landraces showed cultivar reaction to smut differs from cultivar to cultivar, country to country and region to region. This might be attributed to the local adaptation of accessions/varieties to the existing smut pathogen. Therefore, increasing the contribution of Ethiopian local landraces via breeding activity is highly important to improve the profitability of the Ethiopian sugar industry. Olweny *et al.*¹⁷ reported that genetically diverse parents or breeding clones are essential to get high-yielder, disease and insect-resistant cultivars for commercial production.

Weight of smut-free stalk (kg/stalk) and stalks loss (Qt ha⁻¹) for Ethiopian landrace accessions: The highest single mean weight of smut-free stalk (2.49 kg/stalk) and statistically significant difference ($p < 5\%$) from 159, 151, 190, 153, 203 and

Table 2: Ethiopian landraces reactions based on the percentage of infected stools by adopting the scale used by Lemma *et al.*¹⁴

Sugar cane accessions	Disease incidence (%)	Sugar cane accessions on rating	Host response
Mori 60	0.931	1	VHR
159	8.5	4	MR
140	5.677	3	R
151	7.878	3	R
190	4.623	2	HR
153	4.745	2	HR
141	3.666	2	HR
422	6.330	3	R
203	1.321	1	VHR
188	2.542	1	VHR
46	2.245	1	VHR
43	7.576	4	MR
7	2.031	1	VHR
189	1.720	1	VHR
139	1.297	1	VHR
138	2.054	1	VHR
164	3.102	2	HR
4	0.685	1	VHR
157	5.721	3	R
177	5.926	3	R
NCo-334	27.664	9	S
C86-56	0g	0	Immune

VHR: Very highly resistant, HR: Highly resistant, R: Resistant, MR: Moderately resistant and S: Susceptible

NCO334 was displayed by Mori 60, which was statistically at par with 140, 141, 422, 43, 7, 139, 138, 4, 177 and C86/56 (Table 1). According to research findings by Scortecchi *et al.*¹⁸ increasing sugar content in sugarcane crops is closely associated with the height, diameter and the number of stalks, along with sugar accumulation in the stalk. Several resistance accessions were obtained from the Ethiopian land-races collections. All the collections that evaluated Ethiopian land-races accessions were promoted for their further breeding and agronomic evaluations. The drawback of the current field screening techniques is time-consuming and hindered by environmental variability. This drawback might be improved by conducting field screening activities using more efficient, rapid and reliable techniques for the identification of resistant cane varieties that are easily performed under controlled environmental conditions.

CONCLUSION

Sugar cane smut is one of the crucial determining factors to reduce cane yield and productivity. The Use of resistant cane varieties is among the methods to reduce and limit the sugar cane smut expansion under commercialized cane plantations. The current local landraces were evaluated for their resistance reaction to replace the imported inferior materials from abroad where sugar cane diversity is available. According to the current finding, all varieties showed a resistant reaction ranging from R-VHR which implies suitable for production under the integration with other IPM.

SIGNIFICANCE STATEMENT

These Ethiopian landrace's sugar cane varieties showed a promise to replace inferior and safe currency that required incurred to purchase from abroad. The chance of getting resistance variety from the local landrace is high as compared to exotic varieties. Therefore, in this study, several elite sugar cane accessions were identified from the Ethiopian landraces, which provides an immense opportunity for the Ethiopian sugar industry to replace the inferior imported materials that were introduced from abroad. The Ethiopian landraces consist of several important genotypes that are used for sugar breeding owing to their potential adaption to their specific environmental condition and their large genetic variability.

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