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Screening of Sugarcane Varieties against Striga Hermonthica Parasitic Weed Infestation at Beles Sugar Project

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Abstract

Ten selected sugarcane varieties under the same Striga seed concentration were evaluated for their response to striga hermonthica parasitic weed at Beles sugar project. The Striga seed was collected on 150 micron screen sieves while the trash was collected on 250 micron screen sieves separately. Tetrazolium red test was used to detect live Striga seed and the seed was examined through the PCM looking for the red-stained endosperm indicative of viable seed. The amount of Striga seed that was needed to infest each pot was calculated based on the amount of Striga seed that was germinate. All sugarcane varieties were infested into the top 60 cm of soil depth with Striga seed. Treatments were organized in a randomized complete block design with three replications. Striga hermonthica weed infestation was detected on all sugarcane varieties at different infestation level. On average lager number of striga weed populations was attached on susceptible sugarcane varieties SP70/1224 and FGO3-520 while Lower mean attaché striga weed was noted on resistant varieties, NCO-334, N14, B52/298 and C132/81. Susceptible sugarcane varieties displayed stunted growth, tiller mortality and death of stools. In general, the evaluated sugarcane varieties were categorized in Resistance, Moderately resistance and susceptible groups of reaction. The striga infestation did not affect sprout significantly for all sugarcane varieties were under the test. Sprout and tiller population relatively affected by the infestation resulting poor crop stand and stalk formation on susceptible sugarcane varieties. Beles sugar development project has to be periodically monitoring their sugarcane fields planted with susceptible and moderately susceptible sugarcane varieties for striga weed status and take immediate measure. Especial nutrient management should be given for sugarcane varieties planted on poor soil fertility and mechanism of resistance should be further investigated for sugarcane varieties under the production.

Keywords: Striga; Resistance; Susceptible

Introduction

Witchweeds (Striga spp.) are root hémiparasites which cause signifiant yield loss to food crops in Asia and Africa. In Africa, up to 45 million ha of arable land is threatened by thèse weeds [**1**]. Striga' is the Latin word for 'witch'. Buta (Kiswahili), '*Akanchira*' or `*Kitigyn*' in Amharic, '*Desso*' in Afan oromo and other Common names for striga often refer to the word 'witch', presumably because plants attacked by Striga display stunted growth and an overall drought-like phenotype long before Striga plants appear [**2**].

Various species of Striga have been reported in Africa [**3**]. Of the reported species, *S .asiatica* (L) *Kuntze* and S. *hermonthica* (Del) Benth are of economic importance. *S. hermonthica* is the largest and the most destructive of the Striga species weeds in Africa. It parasitizes most important agricultural and commercial crops such as sorghum, maize, millet, rice and sugar cane, as well as pasture and wild grasses, by attaching itself to the roots of the host plant diverting essential nutrients and leaving the host stunted and yielding little or no grain or no biomass, often causing yield losses in excess of 50 % [**4**].

In Ethiopia, sugarcane infestation with Striga are reported from Beles and Wolkayite sugar Project. At Beles, Striga infestation is observed in some of the sugarcane fields affecting sugarcane plants severely ranged from 25 - 100% incidence. Moreover, the volunteer sorghum and some grass species in residential areas were also observed to be highly infested with thèse weed. At Wolkayite Sugar Project also the weed was reported to be problematic.

Various control measures have been recommended to minimize *Striga* related losses. The recommended control measures include mechanical uprooting of the weed, heavy application of nitrogenous fertilizer use of the herbicide 2, 4-D and planting of resistant or tolerant sugarcane cultivars [**5**]. Because of economic constraints and illiteracy most of the available methods for *Striga* control are either expensive or too sophisticated for subsistence African farmers. Resistant crop cultivars have long been proposed as means of reducing losses due to *Striga* under the low-cost input subsistence farming in Africa [**6**]. It is the most economically feasible and environmentally friendly means of Striga control. In East Africa, the most promising new approach to Striga control is the use of resistant cultivars (e.g. of sorghum). Striga resistant cultivars have been bred in a number of crops. However, cultivars with immunity to Striga have not been found in all host crops [**7**].

The host parasite relationship is governed by a series of steps involving stimulation of germination, haustorium initiation, and penetration of the host root, connection to the host xylem and concurrent growth and development [**8**]. A number of resistance mechanisms to *Striga* have been suggested these include i) low stimulant production, ii) mechanical barriers to parasite ingress, iii) in which the crop plants may produce chemical compounds that discourage subsequent development of *Striga* seedlings and iv) hypersensitivity where the host cells surrounding the endophytic part of the haustorium die and preclude further development of the parasite [**8**]. The below-

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ground Striga observations in the assessment of resistance is difficult to make in the field, one has to find other media, such as Petri dishes and pots to study below-ground processes [**8**]. Each genotype possesses its own level of resistance, making it difficult to directly assess the level of tolerance or compare the level of tolerance among genotypes. Furthermore, identification of tolerance requires Striga-free plots as a reference next to infested plots, as each genotype will have its own yield level, which will also be influenced by the specific environment where the screening takes place [**8**].

Yield loss due to striga damage range between 20-80% in Africa, but total crop failure is possible in the worst situation [**9**]. The crop loss in susceptible sugarcane varieties due to infection of the parasite was estimated to 38% in cane yield, 52% in juice brix, 58% in sucrose and 1.89 t/ha in commercial cane sugar [**10**]. However, information related to Striga resistance or tolerance in sugarcane is very inadequate. Therefore, with the above facts, this proposal was initiated with the following objectives:

To identify resistant/tolerant sugarcane varieties to Striga hermonthica parasitic weed infestation

Materials and Methods

Area description

Tana Beles sugar development project is found in Amhara and Benishangul Gumuz regional states 576 Km far from Addis Ababa and about 30 km south-east of Lake Tana where three sugar factories are under construction. Some parts of its cane plantation are found in Benishangul Gumuz Regional State. The land lies within the catchment of the Beles River which flow south-west wards towards the Abay (Blue Nile) River. The project comprises the upper catchments of the Enat Beles River (also called the main Beles) and its main tributary the Abat Beles River. It is located at 11°30’ N and 36°41’ E with an elevation of 1110 m.a.s.l. However, the elevation varies between 1000-1300 m.a.s.l. The Enat Beles originates from the face of an escarpment in the high mountain range at about 2250 m.a.s.l. separating the Enat Beles basin on the west side from Lake Tana. The area receives 1447 mm mean annual rainfall; and mean maximum and minimum temperatures are 32.5 and 16.4°C, respectively. Land form of the Nursery site is slightly concave alluvial basin plane. The cane plantation is irrigated by gravity sprinkler and surface irrigation. The soil of the project area is composed of Nitosols, Luvisols, Cambisols, Fluvisols, and Vertisols. Vertisols, Nitosols, and Luvisols are the most widely spread soils in the study area.

Treatments and Experimental design

The pot experiment was carried out in the open air at Beles sugar development project to compare resistance and performance of selected sugarcane varieties under the same Striga seed concentration. Striga Research Methods Manual used by Berner *et al.* 1997 was adopted to execute this experiment. Ten sugarcane varieties (NCo334, N14, B52/298, C132/81, SP70/1224, C86/12, CIRAD 2011(FG03 520, FG04-466, PSR97-092 and FG04 187) were used as the test materials. Plastic pots, half barrel size, perforated at the bottom was used and each filled with 312 kg of vertisol soil (pH 6.82, EC 3.65 ds m^{-1}, Total N, 0.11% available P 2.38ppm, organic-C 1.43%). The soil was collected from sugarcane fields and mixed uniformly to maintain homogeneity of the likely striga soil seed bank. The standard concentration (3000 to 5000) of viable *Striga* seeds was mixed into the top 60 cm of soil depth in each pot. All sugarcane varieties were infested with *Striga* to see their responses for the infestation.

Treatments were organized in a randomized complete block design with three replications. NPS was applied at a rate of 5.778 gm. /pot manually at time of planting. Nitrogen at rate of 7.563 gm. /pot was applied 2.0 months after planting. The pot was irrigated immediately after application in order to reduce loss of nitrogen through volatilization. Eradication of weeds other than Striga was done by hand, for ease of Striga counting. Three double budded setts were planted in each pot. The setts were protected against termites and other soil borne insects and fungal attack by using the recommended insecticide and fungicide at the time of planting.

Striga Weed Seed Collection

Harvesting *Striga* seed

Heavily infested fields were identified and only the floral heads of the Striga plants were harvested. Only those heads that were mature and have healthy intact (not shattered) capsules were harvested. The harvested heads were collected in paper bags and transported to the drying area **(Figure 1)**.

Drying, cleaning, and storing Striga seed

After harvesting the Striga seed, it was dried in a well-ventilated covered area by, hanging the bags of Striga or the floral heads of Striga were spread on polyethylene sheeting in a wind-sheltered area and was exposed them to the sun to dry. Then, the Striga seed was mixed daily to facilitate even and thorough drying.

After 10 to 14 days of drying, the floral heads were shattered gently on the plastic sheeting to force seed shed tap. The left-over floral head trash was saved to infest “Striga sick plots”. After “threshing” sieves of 250 and 150 micron openings were used to screen the material on the sheeting by passing it through sieving helps to remove most of the plant trash in the seed lot and makes subsequent infestations with the seed

Figure 1: Striga Seed Viability Test Process.

more accurate. The seed was stored in a better and be less susceptible to fungal spoilage area by removing the trash. The Striga seed was collected on 150 micron screen while the trash was collected on 250 micron screen separately.

Standard Striga Seed Rate Estimation

The amount of Striga seed that was needed to infest each pot was calculated by Small scoop that was hold approximately 5 ml of water was prepared and the amount of sand contained one level of scoop was weighing (Sand/scoop). Then, rate calculation was based on the amount of Striga seed that was germinate. To get a good level of infestation and to be economical, a level of 3000 to 5000 viable seed per planting pot was used. Based on the germination percentage, the actual number needed to achieve 1000 viable seed was calculated as 3000 seed (percent viability = seed) pot. The Striga seed weight needed per pot was calculated based on each Striga seed weight 5 X 10^{-6} grams. So, the Striga seed rate needed to infest a single pot was calculated as seed/pot X *5 X* 10^{-6}. The Striga seed weight needed per pot was adjusted (1 - (% trash/100).The total weight of sand needed to infest single pot was calculated at one scoop of Striga-sand mixture per pot **(Figure 2)**.

Striga Seed Viability Test

Tetrazolium red is a more useful indicator of Striga seed viability than germination stimulants; because viable seeds that have not after-ripened and/or not conditioned was not germinate even when treated with a stimulant. Therefore, Tetrazolium red test was used to detect the presence of a dehydrogenase enzyme, which indicates that the Striga seed was alive. After treating the Striga seed with Tetrazolium red, the seed that was produce a red to pink coloration in both the embryo and aleurone layer was identified as viable seed and the seed that was not produce this color change was marked as non-viable seeds.1 g of 2, 3-5 triphenyl Tetrazolium chloride salt was dissolved into 100 ml of water.

The pH of the resulting solution was adjusted in the range between 6 and 8. Acid solutions those with a pH below 7 was neutralized with sodium hydroxide (NaOH). The viability was tested by placing 50-100 Striga seed in a petri dish covered with aluminum foil to exclude light. Enough Tetrazolium solution was added to barely cover the Striga seed. The dish was placed in a warm dark place at 40 C° for 48 h for staining. Depending on ambient temperature staining period was ranged from 2–7 days with incubator **(Figure 3)**. Then the mixture was poured into a funnel lined with a 9 cm filter paper. Water was used to rinse the petri dish and carry any remaining seeds into the funnel and the solution was allowed to drain. To bleach the seed coats and allow the red-stained endosperm beneath to be seen, the filter paper was placed in a clean petri-dish and enough Sodium hypochlorite solution (1% NaOCl) was added to barely cover the seed.

Figure 2: Striga Seed counting and Incubation process.

SP70/1224 FG03-520

Figure 3: Sever Infestation of S. hermonthica Weed on Susceptible Sugarcane Varieties.

The seed was examined through the PCM looking for the red-stained endosperm indicative of viable seed. Finally percent viability was calculated by the formula used by **[11]** as indicated as:

$$\% \text{ viability} = \frac{\text{Total seed} - \text{non germinated}}{\text{Total Seed}} \times 100$$

Striga pot infestation

Considering the smaller size of Striga seeds, and to accomplish soil infestations easily the Striga seeds was mixed with a carrier material to increase volume. Sand was used as a carrier material and only particles of the same size as Striga seed was used. A 212, 150, or 180 micron sieve was used for sieving the sand. Typically this was done by infesting the pot to be planted 7 to 14 days in advance of planting the crop.

Data collection and analysis

Assessment of the response of sugarcane verities to *S. hermonthica* includes, Sprout count at 45 days after planting, Tiller count at 30 days after planting, counting the number of millable stalks at harvest and number of Striga plants around the host plant starting from 45 day after planting was recorded **(Figure 4)**. After recording the Striga weed, it was rouged out to avoid recounting. By using a scale for scoring the infection of Striga was developed. Then, evaluation of the responses sugarcane varieties to *S. hermonthica* was made based on the number of attach Striga weed per sugarcane plant in the pot by adopting the score used by **[11]** in the range indicated below.

Result and Discussions

The Responses of Sugarcane Varieties to Striga Hermonthica Weed Infestation

Pot experiment was carried out in the open air to compare

Figure 4: S. hermonthica Parasitic Weed Infestations on Resistant Sugarcane Varieties.

resistance and performance of ten selected sugarcane varieties under the same Striga seed concentration. Accordingly mean S. *hermonthica* population count per a single sugarcane plant varied among the sugarcane varieties with their respective reaction groups **(Table 3)**. All sugarcane varieties were under the test infested by *S. hermonthica* parasitic weed at different level of infestation. Of the screened sugarcane varieties N14, NCO334 B52/298 and C132/81 showed resistance for *S. hermonthica* weed while varieties C86/56, FG04-187, FG04-466 and PSR97-092 showed Intermediate response and varieties SP70/1224 and FG03-520 showed Susceptible for *S. hermonthica* parasitic weed infestation **(Table 1)**. In contrary with the above finding, reported that sugarcane varietal variation in susceptibility to the Striga weed observed in the fields and the variety B52/298 was observed to be highly susceptible to the Striga weed followed by N14 which was intermediate and NCO 334 remained tolerant. This might be survey approach may not be the appropriate method to categorize sugarcane varieties under natural infestation without the knowledge of the typical Striga seed rate in the soil seed bank. On the other hand the potential of the host plant may not also be expressed under poor crop husbandry practices.

The effect of Striga was first noted two month after planting in trace extent. Severely infested sugarcane varieties exhibited wilting, stunted growth and complete death of stools which contributed to tiller mortality and poor stalk formation. Similarly, Alfonso and Brent 2014 reported that one plant of *S. hermonthica* per host plant is estimated to cause approximately 5 percent loss of yield, and high infestations can cause total crop failure. Among the sugarcane varieties were under the test B52/298, NCO-334, N14 and C132/81were significantly gave better tiller and stalk populations under low Striga weed infestation compared to others and were rated as resistance. Similarly Findings of Mbogo and Osoro found that sugarcane variety Co-617, although allowing some infestation, was highly resistant to *S. hermonthica* while others with nearly equal resistance included KEN-83-1228, KEN-83-538 and KEN-83-1161. Pauls *et al.* 2006 also reported that fourteen elite sorghum lines were evaluated for their resistance to S. *hermonthica* at three locations in Nigeria and Mali; results showed that many of the lines remained

Table 1: Lists of sugarcane varieties used for screening.

SN	Treatments	Variety Descriptions
1	NCO334	Old Variety
2	N14	
3	B52/298	
4	C132/81	Cuba 2011
5	SP70/1224	
6	C86/56	
7	FG03-520	CRAD 2011
8	FG04-466	
9	PSR97-092	
10	FG04-187	

Table 2: Rating Scale for Response of Sugarcane Varieties to Striga Infestation.

Number of striga weed/sugarcane plant	Reaction group
0	Immune
1-2	Resistant
2-3	Intermediate
>3	Susceptible

resistant to *Striga* in all locations with low emerged Striga counts.

The highest numbers of Striga weed count were recorded from sugarcane varieties SP70/1224 followed by FG03-520. As a result, a surplus lethal effect was caused by S. hermonthica parasitic weed infestation on the susceptible host **(Figure 6)**. These effects were reduced in the resistant sugarcane varieties when compared to those in the susceptible varieties. This result agrees with the field test results of Menkir 2006, reported that the higher the number of attached striga plant, the more deleterious the effects of the parasite, where the host is not tolerant. On the other hand, **[12]** also reported that the susceptible line supported a larger number of Striga plants than the resistant line, both on the field and in the screen house.

Among the test sugarcane varieties, on average old sugarcane varieties B52/298, NCO-334 and N14 record lower number of Striga weed per plant with Resistance and Intermediate reaction groups followed by Cuba, 2011 (C132/81, SP70/1224 and C86/56) while, CIRAD 2011(FG03-520, FG04-466, PSR97-092 and FG04-187 showed the highest number of Striga weed per plant with Intermediate and susceptible reaction group. Beside, none of the sugarcane varieties were under the screen showed Immune reaction with zero Striga weed count. Alternatively, infested resistant varieties showed less effects of parasitism than the infested susceptible varieties. This implies that Resistance sugarcane varieties had lower attached Striga weed than susceptible varieties. This might be resistance varieties have inherent physiological/biochemical defense response to parasitism. In line with the above finding, **[13]** reported that, the accumulation or deposition of an unidentified substance at haustoria- host interface in resistant sugarcane varieties as well as poor invasion of the host tissues by the haustoria of the parasite, will likely lead to poor nutrient absorption by the parasite from the host and thus the observed retardation in growth.

Mean attached Striga Weeds Plant on Sugarcane Varieties

On average the number of attached Striga weed per single sugarcane plant was less in resistant varieties compared to susceptible sugarcane varieties **(Figure 5)**. Number of attached Striga weed on susceptible sugarcane varieties exceeds 50% over the resistance varieties. This impies that, the more number of attached Striga weed to the host plant the more infested by the parasite.

In similar manner, reported that attaché S. hermonthica plant on

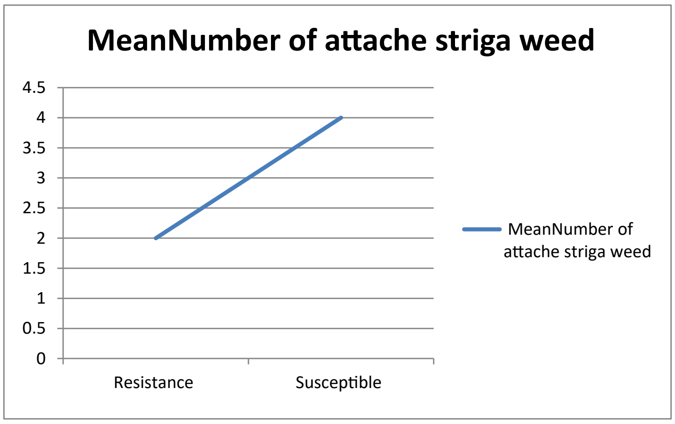

Figure 5: Mean Number of S. Hermonthica Weed on Resistance and Susceptible Varieties.

C86/56 FG04-187

Figure 6: S. hermonthica Parasitic Weed Infestations on Intermediate Sugarcane Varieties.

the resistance line has slow growth rate compared to the one attache to susceptible liens. Large number of attache Striga weeds per plant has implication of high reproduction rate of Striga weeds as the number incérasses. Consistently, Pieterse and Pesch, 1983 reported that, the most frustration information regard to Striga weeds is the reproduction rate and the longevity of the seeds of this weed; roughly 10,000 to 100,000 or more seeds produced per a single Striga plant and when the conditions for the germination are lacking, the seeds can remain viable in soil for a period up to 20 years.

Effect of *S. hermonthica* Weed Infestation on growth paramètres

Significant variation in réaction to Striga infestation were also observed on tiller and stalk population count **(Table 2)**. Sugarcane varieties B52/298, N14, C132/81 and C86/56 revealed the best tiller and stalk population count based on low Striga weed attachments per sugarcane plant and high resistance to S. hermonthica weeds while sugarcane varieties SP70/1224 and FG03-520 revealed the list tiller and stalk population count with high Striga weeds attachement **(Table 4)**. Moreover, the infested resistance sugarcane varieties B52/298, NCO-334, N14 and C132/81 were more vigorous and shows better crop stand when compared to the infested susceptible varieties. In agreement with this, Vasudeva, 1985 reported that the extent to which Striga reduces the growth of its host is highly variable and depends on factors such as host plant genotype, parasite infestation level, and environment. The resistant sugarcane varieties has few Striga attachments, slow parasitic development and higher mortality of attached parasites compared with the susceptible varieties. In line with the above facts, Pieterse and Pesch, 1983 reported that Striga on the susceptible inbred usually penetrated the xylem and showed substantial internal haustorial development.

Table 3: Reaction of Sugarcane Varieties to *S. hermonthica* Weed Infestation.

SN	Treatments	striga count/plant	Reaction Group	Total striga count /pot
1	NCO-334	2	R	85ab
2	N14	2	R	68b
3	B52/298	2	R	47b
4	C132/81	2	R	83ab
5	SP70/1224	4	S	143a
6	C86/56	3	I	88ab
7	FG03-520	4	S	139a
8	FG04-466	3	I	105ab
9	PSR97-092	3	I	103ab
10	FG04-187	3	I	107ab
	Lsd			48.98
	CV%			23.7
	Mean			

Table 4: Effect of Striga Weed Infestation on Sprout, Tiller and Stalk Population.

SN	Treatments	Sprout%	Tiller Pop ('000/ha)	Stalk pop ('000/ha)
1	NCO-334	72.22	1091.0ab	546.0ab
2	N14	72.22	1030.3ab	515.3ab
3	B52/298	72.22	1515.0a	758.0a
4	C132/81	77.78	1015.3ab	507.7ab
5	SP70/1224	61.11	939.3ab	470.0ab
6	C86/56	88.89	1166.7ab	583.7ab
7	FG03-520	50.00	394.1b	197.3c
8	FG04-466	55.56	606.3ab	303.3abc
9	PSR97-092	77.78	863.7ab	432.0abc
10	FG04-187	66.67	1046.3ab	522.7ab
	CV%	21.33	28.7	28.7
	Lsd	48.89	963.28	65.66
	Mean	69.44	966.7	483.00

Haustorial ingress on the resistant inbred was often stopped at the endodermis. Likewise, Aliyu and Emechebe 2006 also reported that parasites able to reach resistant host xylem vessels showed diminished haustorial development relative to those invading susceptible roots. Thèse result suggest that the resistant inbred expresses a developmental barrier and incompatible réponses against Striga parasitism.

There was no significant difference in sprout count for all sugarcane varieties were under the test. This might be attributed to the lethal effect of Striga weed on the host plant was more sever at grand growth and elongation sugarcane growth stage than formative phase. In consistent Menkir 2006 reported that the deleterious effect of Striga on host plant does not depend only on the number of attached plants but also on some intrinsic biochemical and physiological properties of the host or the parasite. In contrary, Eplee and Norris, 1995 reported that one of the main problems is the fact that these root-parasitic weeds cause most of their overall damage to the host crop during their subterranean stage (underground stage). Parker and Riches, 1993 also reported that the subterranean developmental stage of Striga is the most critical and damaging stage to its host which is approximately 75% of the overall Striga damage.

Conclusion and Recommendations

Ten selected sugarcane varieties under the same Striga seed concentration were evaluated for their response to *S. hermonthica* parasitic weed on open air as a pot experiment. *S. hermonthica* weed infestation was observed on all sugarcane varieties were under the test at different level of infestation. On average lager number of Striga weed was attached on susceptible sugarcane varieties SP70/1224 and FGO3-520 while lower mean attaché Striga weed was noted on resistant varieties, NCO-334, N14, B52/298 and C132/81 per a single sugarcane plant. Susceptible sugarcane varieties displayed stunted growth, tiller mortality and death of stools. In general, the sugarcane varieties were screened fall in the reaction groups of Resistance, Moderately resistance and susceptible.

The Striga infestation did not affect sprout significantly for all sugarcane varieties were under the screen. However, sprout and tiller population relatively affected by the infestation resulting poor crop stands and stalk formation on susceptible sugarcane varieties. Therefore based on the above findings, the following recommendations were made.

Beles sugar development project has to be periodically monitoring their sugarcane fields planted with susceptible and moderately susceptible sugarcane varieties SP70/1224 FGO3-520, C86/56, FG04-466, PSR97-092 and FG04187 for Striga weed status and take mediate measure.

The sugar project should also minimize the area coverage of the susceptible sugarcane varieties in the plantation

Rouge out the Striga plant and alternate host of Striga from sugarcane fields before seed setting

Avoid planting of moderately susceptible sugarcane varieties adjacent to highly susceptible sugarcane varieties.

Give especial nutrient management for sugarcane varieties planted on poor soil fertility like Luvi soil type.

Mechanism resistance should be further investigated for sugarcane varieties under the production.

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