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RESEARCH ARTICLE

Evaluation of smut inoculation techniques in sugarcane seedlings

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Abstract Smut disease of sugarcane, caused by the fungus *Ustilago scitaminea* Sydow, can cause considerable yield losses and reductions in cane quality. To investigate the reactions of the seedlings to smut, three different inoculation methods were employed. Data on number of smut whips per stool, disease incidents per population and number of tillers per plot were recorded and analysed. Results showed that screening for smut resistance at the first stage of selection to assess seedling reaction to smut is possible. The wound paste method of inoculation gave better results than the other techniques evaluated.

Keywords *Ustilago scitaminea*, sugarcane, smut, screening, Mtwapa, Kenya, inoculation methods

Introduction

Owing to its vegetative mode of propagation, sugarcane (*Saccharum spp*.) is prone to infection by systemic pathogens. The most effective method of controlling sugarcane diseases is the use of resistant cultivars (Schenck, 1998).The most widely used technique to evaluate for resistance to smut involves immersing sugarcane setts (seed pieces with 1-3 nodal buds) in a teliospore suspension before planting, and counting the number of sori (whips) that develop (Ferreria *et al*., 1989). Resistance is rated on a scale of 1 (highly resistant) to 9 (highly susceptible) (Hutchinson, 1969) based on the percentage of sori produced. If sori are not produced in the first growing season, the plants are grown for a second season and scored again (Lloyd and Pillay, 1980).

To assess smut reaction, researchers typically use a dip inoculation assay in which nodal buds are immersed briefly in a suspension of teliospores, and then planted in a greenhouse. Evaluation can take place in a greenhouse or in the field (Alexander *et al*., 1991).Injection inoculation may induce greater smut infection than dip inoculation, and cultivars can respond differently to the two methods of inoculation (Waller, 1970). The protocol presently used to screen for resistance to covered kernel smut in sorghum is inconsistent and escapes are common (Claflin and Ramundo, 1996). In sugarcane, little work has been done on screening seedlings with smut and evaluating the inoculation methods.

To assess the feasibility of inoculating sugarcane seedlings with smut, and to evaluate the effectiveness of different inoculation techniques, the present study was performed.

Materials and Methods

Agro-ecological zone of the study site

The research was conducted at Sugarcane breeding centre within Coast Agricultural Research Station- Mtwapa(Kenya). The station is located 15km North of Mombasa; Mombasa District; Coast province; center coordinates 03^{0} 56'S-390 44'E; The altitude is 21km. Dominant soil is well drained, very deep dark yellowish brown, friable sandy loam to sandy clay loam. Annual average rainfall in mm of 1,050 – 1,250mm (MoA – NAL, 1987). It is also referred to as coastal lowland zone (CL)

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and semi humid (3) (Boxem *et al*., 1987).

Developing the progeny for inoculation

Co 421 is a variety native from India. It is widely adapted to various agro- ecological zones in Kenyan sugar industry and yields 80-110t/ha (tonnes of cane per hectare). However, the variety has succumbed to smut infection. Co 331 is native to India and is also susceptible to smut. Variety EAK 70-97 was bred during the East Africa Community and selected in Kenya. The variety is smut resistant. Co 945 is native in India. The variety is widely grown in western Kenya for its high sucrose content and resistance to smut.

Two parents involving resistant and susceptible varieties were crossed at Sugarcane Breeding center – Mtwapa. Population one was a cross between Co 421(susceptible) and EAK *70 – 97*(resistant). Population two was a cross between Co 331 (susceptible) and Co 945 (resistant). The seeds were harvested after 21 days following cross pollination, dried under sunny conditions and threshed to recover seed. 2 grams of the seeds were sown in a media containing sandy soil and coconut coir mixed in a ratio of 1: 1 and planted in trays in green house. The seeds germinated after four days and were maintained to ensure fast growth and protection from diseases and pests.

Inoculating seedlings with smut

Seedlings were inoculated in three different ways. 1) Soaking the seedlings in a smut spore suspension (4 grammes of spores per 1 liter of sterile water at 4×10^6 spores/ ml) for 30 minutes. 2) Wounding the seedlings at the bud with a scalpel then applying a paste of smut made at a concentration of 2 grammes of spore for 2 ml of sterile water. 3) Apply a paste of smut at the seedling buds.

Each inoculation method had 30 progenies planted in plastic bags in the green house. Controls of the un-inoculated progenies from the two populations were included. The

Table 1. Analysis of variance on tillers at two, three and four months after planting, survival counts at one, two, three and four months after planting and smut whip count at three and five months after inoculation

Dependent variables	Independent variables and model	D.F	F-value	P-Value	R-Sq	Mean
Tiller count at 2 months	Model	9	3.69	0.014	0.70	28.42
	Population	1	0.16	0.6987		
	Inoculation method	3	7.58	<0.0001		
	Interaction	3	2.22	0.1306		
Tiller count at 3 months	Model	9	4.06	0.0097	0.72	28.62
	Population	1	3.40	0.0865		
	Inoculation method	3	7.10	0.0039		
	Interaction	3	2.50	0.1042		
Tiller count at 4 months	Model	9	2.15	0.0960	0.58	19.41
	Population	1	2.36	0.1468		
	Inoculation method	3	3.08	0.0618		
	Interaction	3	0.73	0.5500		
Survival at 1 month	Model	9	9.79	0.0001	0.86	20.17
	Population	1	4.96	0.0428		
	Inoculation method	3	23.70	<0.0001		
	Interaction	3	3.92	0.0467		
Survival at 2 month	Model	9	6.09	0.0015	0.80	19.20
	Population	1	0.72	0.4100		
	Inoculation method	3	14.55	0.0001		
	Interaction	3	3.43	0.0467		
Survival at 3 month	Model	9	6.09	0.0015	0.80	19.20
	Population	1	0.72	0.4100		
	Inoculation method	3	14.55	0.0001		
	Interaction	3	3.43	0.0467		
Survival at 4 month	Model	9	2.44	0.0654	0.61	15.88
	Population	1	0.67	0.427		
	Inoculation method	3	4.94	0.0152		
	Interaction	3	0.98	0.4305		
Smut whip count at 3 months	Model	9	7.78	0.0014	0.83	0.25
	Population	1	19.38	0.0060		
	Inoculation method	3	7.90	0.0025		
	Interaction	3	7.90	0.0020		
Smut whip count at 5 months	Model	9	1.78	0.062	0.74	1.92
	Population	1	2.82	0.1151		
	Inoculation method	3	1.59	0.2352		
	Interaction	3	7.78	0.0010		

experiment was replicated three times. Maximum and minimum temperatures in the green house were recorded daily. Data collection on seedling reaction to infection began two months after inoculation. Data on number of smut whips per stool, seedling survival and mortality, disease incidents per population and number of tillers per plot was recorded. The data collection continued for six months at two months intervals.

Data analysis

Individual analyses of variance for the green house experiment were performed using the SAS procedure general linear model (GLM) (SAS/STAT, 1994) on plot means of survival, mortality, tillers and disease incidence. Individual ANOVA was done on the inoculation methods and the untreated control. The effect of inoculation on the sugarcane seedlings and the interactions were evaluated. Mean separation and comparisons were tested by Fisher's least significance difference (LSD) test.

Results and Discussion

Inoculation methods and traits measured

On the analysis of variance shown in the Table 1, inoculation methods of soaking, paste and wound paste was significant in influencing survival and tillering and smut whip count at three months. Seedlings that were inoculated had lower survival rates and tillering ability compared to the seedlings that were not inoculated as shown in table 2. For population 1 (Co 421 x EAK70-97), tillering was highest in uninoculated seedling, then followed by soaking, wound paste and paste in that order as shown in Fig. 1. For population 2(Co 331 x Co 945) tillering was highest in uninoculated seedlings, and then followed by wound paste, paste and then soaking as shown in Fig 2. Tillering rate (the rate at which young shoots appear) has been reported to progressively decrease in the field infected sugarcane cultivars (Waller, 1970). Hector *et al*. (1995) commented on the lack of in vitro tillering of sugarcane plantlets in the presence of *U. scitaminea* filtrates as diagnostic feature for smut. In addition to the lack of tillering in smut-inoculated plantlets, susceptible sugarcane cultivars fully express the disease in vitro by producing sori. The high seedling survival registered by seedlings in population two as shown in Table 2 (Co 331 x Co 945) was probably because seedlings in this population were bigger than those in population 1(Co 421 x EAK 70-97). It appears that resistance is expressed only in fully –grown well developed seedlings, since the very young plantlets and weak ones registers very high mortality rates irrespective of resistance ratings.

Plantlet inoculated with smut further suggests that

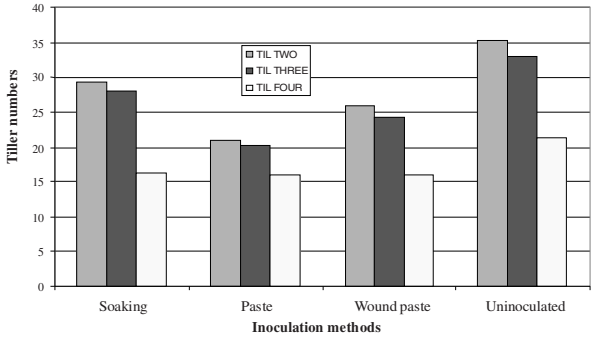

Fig. 1. Effect of inoculation methods of soaking, paste, wound paste and uninoculated on tillering across population 1 (Co 421 x EAK 70-97)

seedlings need to be fully developed before their resistant traits are expressed. In banana, it was also found that the age of tissue- cultured plantlet influenced resistance to nematodes, and very young plantlets did not express all mechanisms of resistance (Elsen *et al*., 2002). High seedlings mortality recorded at four months table 2 was due to sooty mould disease that attacked seedlings in the green house then. Most of the smut inoculated plants died after 5 months following inoculation irrespective of whether or not the sori were produced. The inoculation methods of soaking, paste and wound paste however, was not significant in influencing smut whip count at the age of five months Table 1. Whip production is the most reliable symptom of smut disease in sugarcane, since other features such as the detection of fungal hyphen and *U. scitaminea* DNA may not necessarily be a true indication of plant resistance in inoculation experiments.

Population types were significantly different from each other with respect to survival count at one month after planting and smut whip count at 3 months after planting shown in table 1. Population type were not significantly different from each other in terms of survival at two, three and four months after planting and also tillers at two, three and four months after planting. Wound paste was significantly different from control and soaking in terms of Smut whip count at three months after planting as shown in table 1.

Table 2. Counts on tillers at two, three and four months after planting, mortality at one, two, three and four months after planting and survival at one, two, three and four months after planting

Inoculation method	Counts on various parameter										
	Tillers			Mortality				Survival			
	2	3	4	1	2	3	4	1	2	3	4
Soaking	25	25	17	13	13	13	16	17	17	17	14
Paste	24	25	19	13	15	15	17	17	15	15	13
Wound paste	26	27	16	12	12	12	17	18	18	18	14
Uninoculated	39	38	25	1	3	3	7	29	28	28	23
LSD	7.7 **	7.3 **	7.9 ns	3.6 ***	4.5 ***	4.5 ***	6.5 *	3.6 **	4.5 ***	4.5 **	6.5 *

· Significant at 0.05, ** significant at < 0.01, *** significant at < 0.001, ns not significant

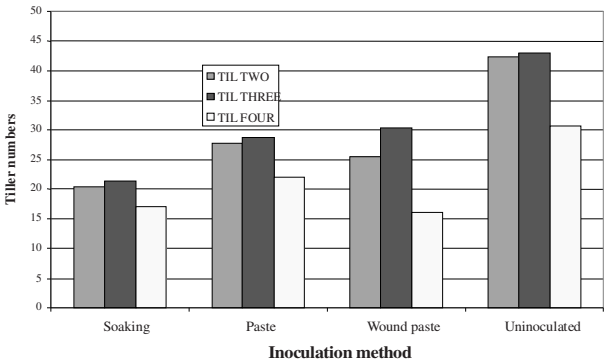

Fig. 2. Effect of inoculation methods of soaking, paste, wound paste and uninoculated on tillering for population 2 (Co 331 x Co 945)

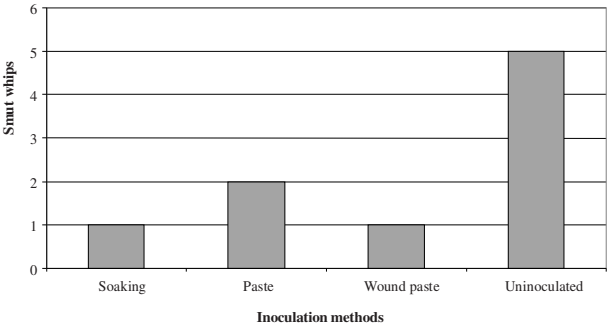

Fig. 3. Effects of inoculation methods of soaking, paste wound paste and uninoculated on smut incidence in population 1 (Co 421 x EAK 70-97)

Laboratory –based disease evaluation in sugarcane dates back several decades when Bock (1964) showed correlation between laboratory and field inoculation for smut disease formation.

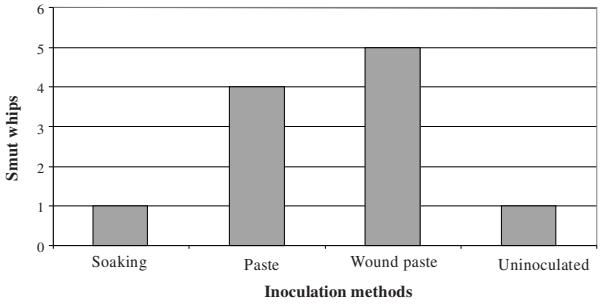

Fig. 4. Effects of inoculation methods of soaking, paste wound paste and uninoculated on smut incidence in population 2 (Co 331 x Co 945)

The correlation analysis shown in table 3 above indicates that neither the survival count nor tillers are correlated to smut incidences. The Smut incidences are independent of tillers and survival. This means that the number of smut whips produced is not related to the survival and tillering rates in sugarcane seedlings. Correlation between survival count and tiller is highly significant. This means that the higher the survival, the higher the tillers. This is conventional because if more seedlings survive they eventually produce more tillers. There is a negative correlation between tillering and smut whip production at three months. This means that tillering does not influence smut whip production in the seedlings. The negative correlation between survival and tillering shown in Table 4 also means that survival of seedlings does not influence smut whip production.

Based on the scores, population 1 (Co 421 x EAK 70-97) registered poor results in terms of whips counted and soral production per inoculation method Fig. 3. There was production of whips on un-inoculated seedlings in this population. This could probably be a mixture of low internal

Table 3. Correlation analysis of tillering at two, three and four months after planting and survival counts at one, two, three and four months after planting and smut whip production at three and five months after inoculation

		Til-2	Til-3	Til-4	Surv-1	Surv-2	Surv-3	Surv-4	Smut-3	Smut-5
Til-2	Correlation	1.0000	0.91804	0.67597	0.81518	.085603	.85603	0.71559	-0.09670	0.10872
	P-value		<.0001	0.0003	<.0001	<0.0001	<.0001	<.0001	.6531	0.6131
Til-3	Correlation	0.91804	1.0000	0.77737	.75319	.82377	0.82377	0.77311	.049370	0.14313
	P-value	<.0001		<.0001	<.0001	<0.0001	<.0001	<.0001	0.8188	0.5046
Til-4	Correlation	0.67597	0.77737	1.000	.55370	0.65595	.65595	0.91338	-0.122241	0.03488
	P-value	0.0003	<.0001		0.0050	.0005	.0005	<.0001	0.5688	.8715
Surv-1	Correlation	.81518	.75319	0.55370	1.0000	.94672	0.94672	0.73498	-0.21188	0.05751
	P-value	<.0001	<.0001	.0050		<0.0001	<.0001	<.0001	0.3203	.7895
Surv-2	Correlation	0.85603	.82377	0.65595	0.94672	1.000	1.0000	0.80867	-0.17305	0.05079
	P-value	<.0001	<.0001	0.0005	<.0001		<.0001	<.0001	0.4187	.8137
Surv-3	Correlation	0.85603	0.82377	.65595	0.94672	1.0000	1.0000	0.80867	-0.17305	0.05079
	P-value	<.0001	<.0001	0.0005	<.0001	<0.0001		<0.0001	0.4187	0.8137
Surv-4	Correlation	0.71559	0.77311	0.91338	0.73498	0.80867	0.80867	1.00000	-0.10292	0.07018
	P-value	<.0001	<.0001	<.0001	<0.0001	<.0001	<.0001		0.6323	0.7445
Smut-3	Correlation	-0.09670	0.04937	-0.12241	-0.21188	-0.17305	-0.17305	-0.10292	1.0000	0.60133
	P-value	0.6531	0.8188	0.5688	0.3203	0.4187	0.4187	0.6323		0.0019
Smut-5	Correlation	0.10872	0.14313	0.03488	0.05751	0.05079	0.05079	0.07018	0.60133	1.0000
	P-value	0.6131	0.5046	.08715	0.7895	0.8137	0.8137	0.7445	0.0019	

resistance of the stalk tissues of individuals in this population or latent infection and inoculation by spores (aero spores) from the nearby-infected seedlings.

For population 2 (Co 331 x Co 945), wound paste method had the highest incidence of smut whip production, followed by paste. Soaking and uninoculated method had the lowest incidence of smut as shown in Fig. 4.

The accuracy of evaluation increased by each crop cycle according to results obtained in a study done in South Africa. Interesting results are therefore expected in the subsequent ratoons. Hence smut levels in the seedlings should be evaluated in the first ratoon as well.

Conclusions

Screening for smut resistance should be done at the first stage of selection, to assess seedling reaction to smut and to avoid carrying large numbers of clones that will eventually be discarded at the advanced stage of selection.

More families should be included in the evaluation process to measure the family effect on smut disease expression in sugarcane seedlings.

The wound paste method led to significantly higher disease levels than the other two treatments. It should be adopted as the inoculation method due to its ease of application and non-injury to sugarcane seedlings.

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