U. of K. J. Agric. Sci. 17(1),131-141, 2009

Assessment of Three Artificial Inoculation Methods for Sugarcane Smut Disease Incited by the Fungus *Ustilago scitaminea* (Syd.)

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Abstract: A field experiment was conducted during 2000/01, 2001/02 and 2002/03 seasons, at the Sugarcane Research Centre, Geneid, Sudan. The objectives of the study were (a) to assess the efficiency and ease of use of three artificial smut inoculation methods; namely, Taiwanese pin-prick (TPPM), dip (DM) and natural spreader-row infection (NSIM) and (b) to evaluate the field response of the tested genotypes to smut. Nine sugarcane genotypes were tested against three checks, in a randomized complete block design with three replications. The mean percentage of smut infection was 2.81, 1.96 and 2.26 for TPPM, DM and NSIM in plant cane (PC); 5.51, 4.39 and 7.4 for the first ratoon (R1) and 6.54, 6.06, 8.66 for the second ratoon crop (R2), respectively. TPPM and NSIM gave slightly high mean percentage of infection values in PC and R1. However, these values were almost similar in R2 for all three artificial inoculation methods tested, indicating the general effectiveness of the three methods in inciting the disease. Therefore, they can all be used; the choice of any one of them should be according to local circumstances and objectives of the study. However, considering the time saved and ease of use, DM is preferred. Four sugarcane genotypes BJ 82105, B 70531, COC 671 and TUC 75-3 had reaction types similar to the resistant checks CO 997 and CO 6806. Three sugarcane genotypes BT 74209, DB 75159 and B 79136 rated either resistant or highly resistant; thus, they are equally suitable for inclusion in the production system. Two sugarcane genotypes, BJ 7938 and BJ 7451, however, had a moderately susceptible reaction type similar to the susceptible check CO 527 and are, therefore, not suitable for commercial production.

Key words: Sugarcane smut disease; *Ustilago scitaminea*; artificial inoculation methods

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INTRODUCTION

Sugarcane smut disease is caused by the fungus *Sporisorium scitamineum* (Syd.) M.Piepenbr., M. Stoll and F.Oberw. (Syn. *Ustilago scitaminea* Syd.), which is primarily spread by air-borne teliospores and infected sugarcane cuttings used as seed cane. It is cosmopolitan in distribution and a major production problem in all 115 sugarcane growing countries in the world except for Papua New Guinea, Fiji Islands and Ecuador, where the disease has not been reported. It was identified as a high risk exotic disease in a pest risk analysis in Australia (Croft and Braithwaite 2006).

The disease substantially reduces the length and girth of cane, number of internodes, moisture content, weight of canes and quality parameters (e.g., brix, pol); also, quantity of juice may be affected (Misra and Ram 1993). Losses in cane yield and sugar due to this disease cannot be easily estimated, but in susceptible varieties cane stands could be reduced to grassy unmillable stalks. Yield and quality losses of between 15% and 20% under moderate levels of disease were reported by Alexander (1995) and Solomon *et al*. (2000). They also indicated that under epiphytotic conditions losses could be enormous. Nasr and Ahmed (1974) documented smut disease in the Sudan in 1964/65 at Guneid sugar scheme. The disease is now found in all sugar estates in the country. No specific studies have been carried out to estimate reductions in yield; although, this is believed to be substantial. Careful inspection, rouging and destruction of infected plants, carried out regularly coupled with hot water treatment regimes of seed cane at 50^0C for 2 hours, can maintain the disease below threshold levels. The use of disease tolerant and/or resistant varieties is the best method of control. Therefore, screening for resistance through artificial inoculation techniques is an indispensable tool and an integral component in the search for resistant varieties, which is the only viable and reliable approach for the long term control of the smut disease in sugarcane.

Several artificial smut inoculation techniques are in use today; namely, wound paste, soil inoculation, sprouted bud inoculation, germinated spore inoculation, spore painting, negative pressure technique, hypodermal syringe inoculation of 10 cm tall shoots (Duttamajumder 2000), inoculation of underground buds at the time of tillering (Yadahalli 2002) and inoculation of meristem region of 1 cm tall shoots (Ferreira 1987) etc. Any of these techniques can be adopted for use according to available logistics. The objective of this study was to test three inoculation methods; namely, Taiwanese pin-prick method, dip method and natural spreader-row infection method, for their efficiency to incite the disease and, their ease of use and to evaluate the field reaction of the tested sugarcane genotypes to smut, under Sudan conditions.

MATERIALS AND METHODS

Field trials were conducted for three consecutive seasons (2000/01, 2001/02 and 2002/03) at the Sugarcane Research Center, Guneid (Lat. 15^{o}N, long. 33^{o}E and approximately 400 m above sea level). The soil is heavy clay and alkaline in reaction with a pH of 8.5 and low in nitrogen, available phosphorus and organic matter. The climate is tropical with low relative humidity.

Land preparation and planting materials

Standard methods of cane seed bed preparation of heavy disking, harrowing and ridging at 1.5 m row spacing were adopted. The entries consisted of twelve sugarcane genotypes, mainly introductions from Barbados, i.e., B 70531, B 79136, BJ 7451, BJ 7938, BJ 82105, BT 72209, COC 671, DB 75159, TUC 75-3, CO 527, CO 997 and CO 6806. The three varieties CO 527, CO 997 and CO 6806 are commercial varieties and were included as susceptible and resistant checks. Three eyed cane seed pieces or setts were prepared from a 9-10 month- old field grown cane crop and used as planting material for each genotype. The setts were artificially inoculated by fresh smut teliospores collected from the cane variety NCO 376, by three protocols of (i) the Taiwanese pinprick (TPPM), (ii) dipping (DM) and (iii) natural spreader-row infection (NSIM) methods, prior to field planting as described hereunder.

(i) Taiwanese pin-prick method (TPPM): Two pin pricks were administered at the base of each bud of each sett after being dipped into a freshly prepared spore paste at a concentration of 2 g spores/5 ml water. The inoculated setts were maintained at room temperature, under plastic (=polythene) bags for 24 hours, before being planted in the field. The plot size was 1 row of 10 m length; the rows were 1.5 m apart and 20 cane setts were planted in each plot. The trial was laid in a randomized complete block design with three replications.

(ii) Dip method (DM): The plot size, number of setts per plot and field layout was as in TPPM. The seed setts were inoculated by dipping into a smut spore suspension at a concentration of 1 g smut spores/litre of water for 15 to 20 minutes. The setts were also maintained under plastic (=polythene) bags and planted in the field after 24 hours as in TPPM.

(iii) Natural spreader-row infection method (NSIM): The plot size was 2 rows of 10 m length. Twenty setts were planted per row (40 setts per plot); the rows were 1.5 m apart. A highly susceptible sugarcane variety, NCO 376, was inter-planted between each of the two rows of the test genotypes to act as spreader row and a steady source of smut inoculum throughout the growing season. The trial was also laid in a randomized complete block design with three replications. Data were collected on disease incidence parameters, i.e., number of fungal sori (whips) and number of diseased and healthy stools. Counts started at first whip emergence 60-90 days after planting and continued at monthly intervals for six to eight months.

Evaluation of resistance: Reaction of genotypes to the smut disease, as a criterion for resistance, was determined from the percentage of disease incidence at the end of the second ratoon crop and rated on a numerical scale of 1-9, where 1=highly resistant and 9=highly susceptible (Satya and Beniwal 1978).

RESULTS AND DISCUSSION

The three inoculation methods used were all effective in inciting the smut disease of sugarcane in the genotypes tested (Table 1). The mean percentage of stool infection for TPPM, DM and NSIM were 2.81, 1.96, 2.26 for plant cane; 5.51, 4.39, 7.4 for the first rations (R1) and 6.54, 6.06, 8.66 for the second ration (R2), respectively. At the end of R2 stage, four genotypes, B 70531, BJ 82105, COC 671 and TUC 75-3, rated highly resistant and had reaction types similar to the resistant checks CO 997 and CO 6806; therefore, they were considered suitable for further propagation. Three genotypes, B 79136, BT 74209 and DB 75159, rated inconsistent, between resistant and highly resistant, indicating a good level of tolerance and they too can, thus, be propagated with confidence. Two genotypes BJ 7938 and BJ 7451 had reaction types of either resistant or moderately susceptible similar to the susceptible check CO 527 and are to be discarded as unstable materials.

While these results tentatively indicate the general effectiveness of all the tested methods in inciting the disease, the dip method would be most preferable given the ease and uniformity of the inoculation procedure. In addition, disease escape, due to structural resistance barriers as in NSIM, and damaged or killed buds as may occur in TPPM, are completely avoided. Moreover, a large number of samples can be easily handled in a short period of time. Yadahalli (2002) reported that inoculation below the buds by hypodermic syringe is the best method; also, Abo and Okusanya (1996) maintained a similar view. However, in our experience, TPPM proved to be time consuming, labour intensive and not suitable for large number of samples. It can, however, be used for smaller number of samples and only when measuring physiological or biochemical resistance since its procedure destroys morphological resistance structures. This argument is in agreement with that of Mohanraj and Padmanaban (1987) who, also, reported that TPPM method is not suitable to determine clonal resistance in the field. Whittle and Walker (1982) also suggested that plant cane data, especially for TPPM, should be carefully interpreted as it may tend to over-estimate susceptibility. The

methods advanced by Duttamajumder (2000) and Ferreira (1987), though reported to be superior to DM, are even much more tedious and impractical under Sudan conditions than TPPM. Elsewhere, El-Kholi (1996) reported DM and wound paste method (WPM) as best for testing smut resistance in sugarcane; however, he too preferred DM over WPM for reasons of time saving.

CONCLUSIONS

Based on the results of this study, the following conclusions can be drawn:-

1. DM is the best method for artificial smut inoculation trials and suitable for use under Sudan conditions.
2. Both TPPM and NSIM methods are effective in artificially inducing the infection and can be used for special study purposes such as the determination of physiological resistance in sugarcane genotypes (TPPM) and where a highly sensitive genotype for use in spreader-row is readily available (NSIM).
3. Four genotypes; namely, B 70531, BJ 82105, COC 671 and TUC 75-3, had highly resistant reaction types as the resistant check variety CO 6806 and CO 997 and are suitable for production.
4. Three genotypes, B 79136, BT 74209 and DB 75159, rated either resistant or highly resistant and are considered tolerant materials, hence suitable for use.
5. Two genotypes, B 7938 and BJ 7451, had moderately susceptible reaction type as the susceptible check CO 527 and are, thus, not suitable for the production system.

Table 1. Response of some sugarcane genotypes to three methods of inoculation by the smut disease. Guneid, Sudan (2001-03)

Genotype	Percent stool infection (PC: 2000/01)				Percent stool infection (R1: 2001/02)				Percent stool infection (R2: 2002/03)			
	TPPM	DM	NSIM	Mean smut infection, rating and reaction type	TPPM	DM	NSIM	Mean smut infection, rating and reaction type	TPPM	DM	NSIM	Mean smut infection, rating and reaction type
B 70531	0.0	0.0	1.4	0.46 (1) HR	0.0	2.01	1.27	1.09 (1) HR	0.0	1.07	3.2	1.42 (1)HR
B 79136	1.78	4.59	1.4	2.59 (1) HR	7.4	3.15	2.93	4.49 (2) R	2.6	2.37	2.4	2.45 (1)HR
BJ 7451	2.54	0.0	7.9	5.17 (2) R	9.33	7.6	23.3	13.4 (5) MS	10.3	11.7	16.0	12.6 (5)MS
BJ 7938	5.42	4.2	6.13	5.25 (2) R	16.7	11.1	20.7	16.2 (5) MS	16.4	14.3	21.7	17.5 (5)MS
BJ 82105	0.0	0.0	0.0	0.0 (1) HR	0.0	0.0	2.07	0.69 (1) HR	0.0	0.34	1.03	1.37 (1)HR
BT 74209	0.0	1.3	0.5	0.6 (1) HR	0.6	9.0	0.80	3.47 (2) R	2.36	1.85	1.1	1.77 (1)HR
COC 671	2.35	0.0	1.23	1.19 (1) HR	1.87	0.9	3.57	2.11 (1) HR	5.3	2.57	0.0	2.62 (1)HR
DB 75159	0.0	0.0	0.0	0.0 (1) HR	1.87	2.8	0.4	1.69 (1) HR	0.0	7.36	13.5	6.95 (3)R
TUC 75-3	2.92	3.7	0.6	2.41 (1) HR	0.57	1.07	0.3	0.64 (1) HR	2.2	0.73	0.0	0.97 (1)HR
CO 527	18.62	8.3	7.45	11.42 (4) R	26.5	14.9	30.0	23.8 (5) MS	39.3	28.8	40.5	36.2 (7)HS
CO 997	0.0	0.0	0.48	0.16 (1) HR	1.2	0.0	3.4	1.53 (1) HR	0.0	1.53	4.6	2.04 (1)HR
CO 6806	0.0	1.39	0.0	0.46 (1) HR	0.0	0.0	0.0	0.0 (0) HR	0.0	0.0	0.0	0.0 (1)HR
Mean	2.81	1.96	2.26	2.34 (1)	5.51	4.39	7.40	5.76 (2)	6.54	6.06	8.66	7.08 (3)

1= highly resistant (HR) (0-3% infection); 2-4 = resistant (R) (4-12% infection); 5 = moderately susceptible (MS) (13-25% infection); 6 = Susceptible (S) (26-35% infection); 7-9 = highly susceptible (HS) (>36% infection)

TPPM = Taiwanese pin-prick method; DM =dip method; NSIM = natural spreader-row infection method; PC = plant cane; R1 = first ratoon; R2 = second ratoon

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مجلة جامعة الخرطوم للعلوم الزراعية: المجلد السابع عشر– العدد الاول، 2009م

تقييم كفاءة ثلاث طرق لإحداث عدوى صناعية في قصب السكر بمرض التفحم المسبب بالفطر(Syd.) *Ustilago scitaminea* تحت ظروف السودان

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موجز البحث: أجريت تجربة حقلية لثلاثة مواسم متتالية (2001/2000 و 2002/2001 و2003/2002) بمزرعة بحوث قصب السكر بالجنيد أولا لإختبار كفاءة وسهولة إستخدام ثلاث طرق لإحداث عدوى صناعية بمرض التفحم في قصب السكر وهى طريقة الثقب التايوانية و طريقة الغمر وطريقة الزراعة وسط خطوط من صنف مصاب بالمرض، وثانيا لتقييم مستوى مقاومة الطرز الوراثية المختبرة للإصابة بمرض التفحم . أختبرت تسعة طرز وراثية من قصبة السكر مقارنة بثلاثة أصناف كشاهد. نفذت التجربة بتصميم القطاعات كاملة العشوائية بثلاثة مكررات .كانت متوسطات النسب المئوية للإصابة بالتفحم: في القصب الغرس 2.81 و1.96 و2.26 وفي الخلفة الأولى 5.51 و 4.39 و 7.4 ، وفى الخلفة الثانية 6.54 و 6.06 و8.66 لطريقة الثقب التايوانية وطريقة الغمر وطريقة الزراعة وسط خطوط من صنف مصاب على التوالي . طريقة الثقب التايوانية وطريقة الزراعة وسط خطوط من صنف مصاب أبدتا ارتفاعا طفيفا في متوسطات مستوى الإصابة في القصب الغرس والخلفة الأولى، بينما كانت مستويات الإصابة متشابهة في الخلفة الثانية للثلاث طرق المختبرة و المستخدمة لإحداث العدوى الصناعية بالمرض، مما يدل على إمكانية إستخدام الطرق الثلاث، وان إختيار أى من هذه الطرق يتوقف علي ظروف التجربة وأهداف الدراسة. ولكن لكسب الوقت ولسهولة التطبيق تعد طريقة الغمرهى الأفضل. ابدت الطرز الوراثية

BJ 82105 و B 70531 و COC 671 و TUC 75-3 مقاومة للإصابة بمرض التفحم مشابهة لمقاومة الأصناف القياسية CO 6806 و CO 997 . أما الطرز الوراثية DB 74209 79136 و BT 75159 فكانت إما مقاومة أوعالـية المقاومة مما يجـعـلهـا مناسبة للإنـتاج التجارى بالنسبة للطرز الوراثيـة BJ 7938 و BJ 7451 فأنها متوسطة القابلية للإصابة بالتفحم وبالتالي فهى غير مناسبة للإنتاج التجارى .