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Evaluation of Resistance in Introduced Sugarcane Genotypes to Smut (*Ustilago scitaminea* Sydow) Disease

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Abstract: Fourteen sugarcane (*Saccharum* spp.) genotypes were evaluated for resistance to the smut fungus (*Ustilago scitaminea* H. Sydow) disease of sugarcane in a field trial for two consecutive seasons (2006/07 and 2007/08) at Guneid, Sudan. The commercial cultivars CO 527, CO 997 and CO 6806 were used as checks. The setts were inoculated artificially by two methods: (a) the Taiwanese pin-prick method (TPPM) and (b) dipping method (DM). Data were analyzed by the weighed complete linkage cluster algorithm (CLINK). The results showed that the 17 genotypes tested could be grouped into 4 clusters in DM and 5 clusters in TPPM. In both categories, resistance was weakest in cluster I and strongest in cluster IV in DM and cluster V in TPPM. The resistance characters also gradually increased from cluster I to IV and cluster I to V in DM and TPPM, respectively. Meanwhile, on the conventional scale, 10 genotypes were rated as highly resistant or having resistant reactions similar to those of the resistant checks CO 6806 and CO 997. They can, thus, be propagated in commercial fields. Two genotypes; namely, D 9227 and FR 9973, had smut grades of S5 corresponding to medium susceptible reaction type and are considered unstable and consequently not suitable for further propagation. Another two genotypes, FR 99204 and FR99314, were rated between highly resistant and resistant and the cluster discrimination identified these genotypes as suitable, due to a strong bud resistance, and can thus be used. Also, all the 10 resistant genotypes were classified as resistant by the clustering method and were grouped in clusters III and IV in DM and clusters III, IV and V in TPPM.

Key words: Sugarcane; *Ustilago scitaminea*; resistance; CLINK cluster analysis

INTRODUCTION

The inflorescence smut disease of sugarcane (*Saccharum* spp.), also known as culmicolous smut, is a condition incited by the fungus *Ustilago*

scitaminea H. Sydow {Syn. *Sporisorium scitamineum* (H. Sydow) M. Piepenbring}. The disease is usually easily identified in the field by a characteristic long whip-like, curved, silver-grey to black pencil-thick appendage that develops at the apex of the infected plant. The whip can be a few centimeters to 1.5 m in length; longer whips often curve downwards. Smut is now found in most of the sugarcane growing countries in the world (Singh *et al*. 2004).

Of the various sugarcane diseases, smut causes the greatest yield losses and is by far the most difficult to control as all commercial sugarcane varieties are polyploid hybrids of several *Saccharum* species. Thus, genetic resistance does not follow the strict gene for gene pattern as in some fungal pathogen-host interactions (Vanky 2001; Schenck *et al*. 2005). The disease reportedly reduces the length and girth of cane, number of internodes, moisture content and weight of canes (Solomon *et al*. 2000). Precise data on actual losses in cane yield and sugar due to this disease are not available and assessments cannot be easily rendered (Antoine 1961). The disease can cause yield losses greater than 50% in susceptible varieties and can make ratoon crops unprofitable. Losses are, therefore, extremely variable; many workers estimated it to be in the range of 60%-70% (Fawcett 1942; Raga *et al*. 1972; Alexander 1995 Solomon *et al*. 2000).

The disease first appeared in Sudan at Guneid Sugar Estate in 1964/65 (Nasr and Ahmad 1974). Now, it is found in all the sugar estates in the Country. No specific studies have been carried out to estimate reductions in yield; however, this is believed to be substantial since infection levels of up to 90% were common in susceptible clones (Nasr and Ahmad 1974). However, careful crop inspection, rouging and destruction of infected plants, carried out regularly, can maintain the disease below threshold levels. The use of disease tolerant and/ or resistant varieties is so far the best and only sustainable method of control. Hence, this trial was initiated to identify sugarcane genotypes with suitable resistance or tolerance to the smut disease.

MATERIALS AND METHODS

The experimental site/ location

This study was conducted at the Sugarcane Research Center, Guneid (latitude 15^{o}N, longitude 33^{o}E and about 400 m above sea level) for two consecutive seasons (2006/07 and 2007/08). The soil at the experimental site is vertisols (about 64% clay, 0.09% N and 2-8 ppm available P) and alkaline in reaction (pH=8.2). The climate of the locality is semi-arid with low relative humidity and mean annual rainfall of about 112 mm, falling mainly in July and August.

Seedbed preparation: Conventional methods of sugarcane seed bed preparation of heavy disking, harrowing and ridging at 1.5 m row spacing were adopted as per the standard procedures.

Test materials and inoculation methods

The entries were fourteen sugarcane genotypes; namely, D 9227, BJ 82105, FR 99379, FR 99314, FR 99204, FR 9821, FR 9949, CO 997, BBZ 8063, D 90157, FR 9973, FR 9641, B 871294, CP 88-1762, CO 6806, CO 527 and FR 99348. They were introductions from West Indies and France. The three commercial cultivars CO 527, CO 997 and CO 6806 were used as checks. Single-eyed cane seed setts were prepared from 8-10 months old field grown cane crop of each genotype. The setts were then artificially inoculated by fresh smut (*Ustilago scitaminea* Sydow) teliospores collected from the cane variety NCO 376 by (i) the Taiwanese pin-prick method (TPPM) and (ii) dipping method (DM).

Taiwanese pin-prick method (TPPM): The base of buds of each seed sett was pricked twice by a hypodermic syringe after being dipped into a freshly prepared paste of pure spores (1-1.5 g spores/10 ml water). The inoculated setts were maintained at room temperature in the laboratory under moist conditions for 24 hours. Thereafter, the setts were planted in the field. The plot size was one furrow of five metres length. Furrows were spaced 1.5 m apart, and 20 single-bud cane setts were planted in each plot as double setts on 4 March 2007. The trial was laid out in a randomized complete block design with three replications.

Dipping method (DM): Plot size, number of setts per plot and field layout was as in TPPM. The seed setts were inoculated by dipping in

a spore suspension at a concentration of 1g smut spores/ litre of water for 15-20 minutes. The setts were conditioned, and the experiment designed as in TTPM.

Data collection

Data were collected on the following:-

(a) Infection parameters; namely, disease incidence and number of whips, with counts beginning at first whip emergence 60-90 days after planting (DAP) and continuing at monthly intervals for about six months. The trial was ratooned at 8 months for plant cane and 6 months each for the successive ratoon crops.

(b) Epidemiological parameters; namely, latent infection period in days (LIP/D = the period from inoculation to first disease symptom expression), sustained disease duration in days (SDD/D = the time from first disease symptom expression or LIP/D to harvest), cumulative number of whips per feddan (CNOW/F) and the area under disease progress curve (AUDPC). The formula for AUDPC according to Xu *et al*. (2004) is as follows:-

$$AUDPC = (SI_1 + SI_2) / 2 \times (T_2-T_1)$$

where

SI_1 and SI_2 = stalk infection and / or cumulative number of whips

T_1 and T_2 = the adjacent or any two investigation times

Data on epidemiological parameters were taken only for the plant cane crop stage, which was harvested at the age of eight months, because spores are known to lose viability very rapidly in about 2 to 3 months in wet soils, according to Luthra *et al*. (1938) and Leu (1969). Therefore, this will imply that infection after eight months under field conditions cannot be attributable to the original spores used in the inoculation procedure, but rather to external spore sources; namely, auto infection (=spores from infected plants within the same field) and allo infection or spores from neighbouring fields.

Resistance to smut disease was evaluated based on the varietal reaction types to the disease derived from percentages of the mean disease incidence calculated on clump/stool basis. This was rated on a 1-9 scale

or smut grades (S) where 1 = highly resistant and 9 = highly susceptible, according to the resistance scale of Satya Vir Beniwal (1978). Later, the infection indexes LIP/D, SDD/D, CNOW/F and AUDPC were weighed by standardizing to '*z-scores'* prior to analysis by the hierarchical complete linkage cluster algorithm (CLINK) and compared with the smut grade methods for consistency (Romesburg 1984; Schonlau 2002; Xu *et al*. 2004). The computer programme Genstat Discovery Edition 3 was used in running the analysis.

RESULTS AND DISCUSSION

A symptomatic cane shoot with a typical smut whip at the apex of an infected stalk and whip on side shoots is depicted in Figure 1. Table 1 shows, that at the end of the second ratoon crop stage, the reaction types for DM and TPPM inoculation methods were as follows: Nine genotypes rated highly resistant (HR), five genotypes rated resistant (R) and three genotypes rated medium susceptible (MS) in the dip-inoculated trials. And, eight genotypes rated HR, four genotypes rated R and five genotypes rated MS in TPPM. All genotypes with HR and R reaction types were considered suitable for further propagation and genotypes with MS, susceptible (S) or highly susceptible (HS) reaction types were rejected as unsuitable for any commercial utilization.

A B

Figure 1. Smut symptoms on sugarcane: (A) Mature whip on apex of cane shoot and (B) *Lalas* or whip on side shoots of cane (note the curvature of whips)

Ten genotypes; namely, BJ 82105, FR 99379, FR 9821, FR 9949, BBZ 8063, D 90157, FR 9641, B 871294, CP 88-176, FR 99379 and FR 99348, were HR or R with disease grades (S) of between S1 and S4 (Table 1). This corresponds to highly resistant or resistant reaction types, which is similar to those of the resistant checks CO 6806 and CO 997. This is a good performance and indicative of a suitable degree of resistance/tolerance to the smut disease, and they can thus be propagated with confidence in commercial fields. However, two genotypes; namely, D 9227 and FR 9973, showed smut disease grades of S5 corresponding to MS reaction type similar to that of the susceptible check variety CO 527. Genotypes with an MS reaction type or lower are considered unstable to the disease as they can inevitably support local epiphytotics and are, therefore, considered unsuitable.

Genotypes FR 99204 and FR 99314, however, had inconsistent reaction types of HR and R in DM and MS in TPPM. This rather unusual behaviour can be due to the presence of exceptionally tight bud scales in these genotypes, leading to a highly effective structural resistance barrier. On the other hand, the removal of these structures as was the case in TPPM procedure rendered them completely susceptible, an indication of a weak internal resistance. They are, therefore, safe to use provided that their bud scales are not damaged during the handling and transportation of seed cane. Also, these genotypes can only be infected by soil borne inoculum through damaged buds, but are completely resistant to wind borne aerial spores. The cluster discrimination of genotypes, depicted in Table 2, also grouped these genotypes in cluster IV (highly resistant) in DM and cluster I (highly susceptible) in TPPM. This further confirms and characteristically indicates that they have a strong bud resistance, but a relatively weak physiological resistance. Luthra *et al*. (1941) also stipulated that infection does not necessarily reduce yields in some varieties; thus, depending on local circumstances, some genotypes with MS reaction type that have outstanding agronomic characteristics can be cautiously utilized commercially. This viewpoint is, also, strongly supported by the work of Whittle and Walker (1982) in Guyana who reported that, certain genotypes with MS reaction type maintained good yields under improved management. It is, therefore, evident that they

were probably referring to genotypes with similar behaviour to that of FR 99204 and FR 99314.

Table 1. Performance of sugarcane genotypes to smut disease at the end of the second ratoon crop inoculated by TPPM and DM

Code	Genotype	LIP/d (PC)	SDD/d (PC)	SI (%) (R2)	CNOWF (x1000) (R2)	AUDPC (PC)	Disease grade (S)	Reaction type (R2)
				Inoculation by TPPM				
1	D 9227	75.67	143.33	13.89	6.38	44.61	5	MS
2	BJ 82105	100.33	118.67	3.19	0.22	0.60	1	HR
3	FR 99379	109.67	109.33	11.82	5.18	121.88	4	R
4	FR 99314	75.67	143.33	13.88	5.85	11.12	5	MS
5	FR 99204	35.00	184.00	13.04	3.48	0.00	5	MS
6	FR 9821	70.00	149.00	2.77	1.96	0.00	1	HR
7	FR 9949	105.00	114.00	9.71	2.36	44.44	4	R
8	CO 997	20.33	198.67	2.20	0.09	17.65	1	HR
9	BBZ 8063	95.00	124.00	2.72	0.22	0.00	1	HR
10	D 90157	95.00	117.33	4.76	1.34	11.48	2	R
11	FR 9973	99.67	119.33	14.91	7.34	45.51	5	MS
12	FR 9641	75.67	143.33	6.36	3.34	9.61	2	HR
13	B 71294	219.00	0.00	0.00	0.00	0.00	1	HR
14	CP 88-1762	88.33	130.67	2.10	0.68	2.73	1	HR
15	CO 6806	219.00	0.00	2.38	0.53	0.50	1	HR
16	CO 527	100.30	118.67	18.69	6.04	25.20	5	MS
17	FR 99348	105.00	114.00	6.21	4.54	34.20	2	R

Table 1. Cont.

Code	Genotype	LIP/d (PC)	SDD/d (PC)	SI (%) (R2)	CNOWF (x1000) (R2)	AUDPC (PC)	Disease grade (S)	Reaction type (R2)
				Inoculation by DM				
1	D 9227	205.00	35.00	14.87	5.85	0.00	5	MS
2	BJ 82105	195.00	15.00	1.40	0.09	0.00	1	HR
3	FR 99379	30.33	188.67	2.17	0.56	0.00	1	HR
4	FR 99314	30.33	188.67	5.80	0.75	0.00	2	R
5	FR 99204	65.33	153.67	2.10	1.12	0.00	1	HR
6	FR 9821	20.33	198.67	2.89	0.19	0.00	1	HR
7	FR 9949	39.67	179.33	4.86	0.41	0.00	2	R
8	CO 997	110.00	109.00	1.46	0.19	0.50	1	HR
9	BBZ 8063	43.33	175.67	1.37	0.12	0.00	1	HR
10	D 90157	146.33	72.67	7.24	1.21	1.99	3	R
11	FR 9973	85.00	134.00	13.78	6.71	9.58	5	MS
12	FR 9641	35.00	207.33	3.72	1.21	7.85	1	HR
13	B 871294	65.00	187.33	2.77	1.24	0.99	1	HR
14	CP 88-1762	39.67	179.33	4.43	0.93	1.99	2	R
15	CO 6806	205.00	35.67	0.92	0.22	0.00	1	HR
16	CO 527	195.00	15.00	14.40	3.90	0.00	5	MS
17	FR 99348	35.00	184.00	6.52	2.89	1.00	3	R

TPPM = Taiwanese pin-prick method; DM = dipping method; LIP/d = latent infection period in days; SDD/d = sustained disease duration in days; SI (%) = percentage of disease infection; CNOW/F = cumulative number of whips/ feddan; AUDPC = area under disease progress curve; HR = highly resistant; R = resistant; MS = medium susceptible; PC = plant cane; R2 = data taken at the end of second atoon stage; *Figures are means of three replicates.

Table 2. Differential resistance to smut and distribution of the tested sugarcane genotypes in the various clusters at the end of the second ratoon crop

Cluster No.	Cluster discrimination of sugarcane genotypes	
	Inoculation by DM (Fig.1 A)	Inoculation by TPPM (Fig.1 B)
I	CO 527, D 9227	FR 99314, D 9227, CO 527, FR 9973, FR 99204
II	FR 9973	FR 99379
III	CO 6806, BJ 82105, CO 997, D 90157,	CP 88-1762, BBZ 8063, FR 9821, BJ 82105, CO 997,
IV	CP 88-1762, B 871294, FR 9949, FR 99379, FR 99314, FR 9821, BBZ 8063, FR 99204, FR 99348, FR 9641	FR 9641, FR 99348, D 90157, FR 9949,
V	-	CO 6806, B 871294

DM = Inoculation by dip methods; TPPM = inoculation by Taiwanese pin-prick methods

Nevertheless, it should also be noted that smut is essentially a disease of dry areas; hence, in the more humid conditions of Guyana and the Central American basin, genotypes with MS reaction type are expected to do fairly well even if infected with smut. This is because the high precipitation in those areas effectively removes stresses associated with water in-availability and, thus, maintains the vigour of the plants. Also, smut spores are known to lose viability very rapidly under moist field conditions (Luthra *et al*. 1938; Leu 1969). This tends to lower the effective spore inoculum levels. However, on the other hand, these water related stresses are apparent at some times of the year in most irrigated fields. Thereafter, under the irrigated and semi-arid climatic conditions of Sudan, the water related stresses are effectively intact. Therefore, the severity of smut disease on the crop is expected to be more profound, thus justifying the unsuitability of some MS rated genotypes.

The phylogenetic trees show that the 17 genotypes tested were grouped into 4 clusters in DM and 5 clusters in TPPM (Figure 2). Cluster discrimination of genotypes in both DM and TPPM indicated that resistance was weak in the lower clusters, but this gradually increased to strong in the higher clusters. The 10 genotypes that were identified as suitable by the conventional rating system are, also, located in clusters III and IV in DM and clusters III, IV and V in TPPM.

Furthermore, by comparing Figure 2 A and 2 B we can deduce that the resistant check varieties CO 997 and CO 6806 and test genotypes (FR 9821, B 871294, BBZ 8063, BJ 82105, FR 9641 D 90157, FR 9949 and CP 88-1762) were all bracketed in clusters III and IV in DM (tight bud scale resistance) and clusters III and IV in TPPM (high physiological resistance). Genotype B 871294 and the check variety CO 6806 were, however, bracketed in cluster V in TPPM (very high physiological resistance). On the other hand, genotypes FR 9821, B 871294, CO 997, BBZ 8063, BJ 82105, CO 6806 and CP 88-1762 were bracketed in cluster III and IV in DM and Cluster III, IV and V in TPPM. This shows that there is a relatively strong bud scale resistance and strong physiological resistance as well, and are, therefore, excellent genotypes. Accordingly, these genotypes were also rated either HR or R, using the single parameter of percentage disease infection.

Table 2 shows the differential resistance to smut and varietal distribution in the different clusters for the two inoculation methods. The differences in the distribution of varieties in the various clusters are due largely to differences dictated by the varying strengths of the structural and biochemical/ physiological resistance mechanisms already discussed and modifications imposed by the environmental component. The clustergrams in Figure 2 have tentatively illustrated these relationships. Furthermore, the target of cluster analysis is, usually, to aid in easy visualization and understanding of the relatedness (similarity) or un-relatedness (dissimilarity) between the different cases or genotypes, utilizing the additive effects of the resistance parameters or objects indexed for the test (Xu *et al*. 2004).

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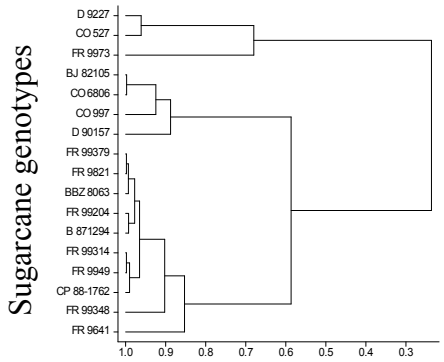
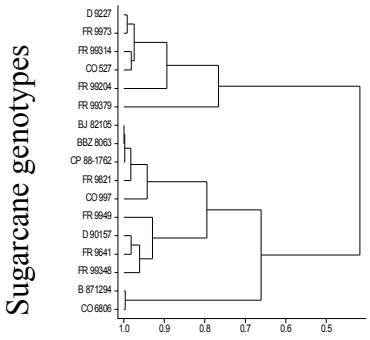


(A) Euclidean distance, e_{jk}

Cluster I: D 9227; CO 527; II: FR 9973; III: CO 6806, BJ 82105, CO 997, D 90157; IV: CP 88-1762, B 871294, FR 9949, FR 99379, FR 99314, FR 9821, BBZ 8063, FR 99348, FR 99204, FR 9641



(B) Euclidean distance, e_{jk}

Cluster I: D 9227, FR 99314, CO 527, FR 9973, FR 99204; II: FR 99379; III: CP 88-1762, CO 997, BJ 82105, FR 9821, BBZ 8063; IV: FR 9641, FR 99348, D 90157, FR 9949; V: CO 6806, B 871294,

Figure 2. Weighed complete linkage cluster algorithm phylogenetic trees for the 17 sugarcane genotypes inoculated by (A) dip (B) pin-prick methods

CONCLUSIONS

The sugarcane genotypes BJ 82105, FR 99379, FR 9821, FR 9949, BBZ 8063, D 90157, FR 9641, B 871294, CP 88-1762, and FR 99348 are HR or R to smut as the resistant checks CO 6806 and CO 997. Thus, these ten genotypes can, therefore, be used in the production system. With good seed cane handling practices, in which buds do not sustain injury, FR 99314 and FR 99204 can also be used successfully in the production system. D 9227 and FR 9973 had an MS rating in both DM and TPPM and, therefore, they are deemed unsuitable materials, thus expansion in production of these genotypes is not advisable.

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تقويم بعض اصناف قصب السكر لمقاومة مرض التفحم السوطى الذى يسببه الفطر *Ustilago scitaminea* Sydow

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موجز البحث: تم تقويم أربعة عشر طرزاً وراثية من هجن قصب السكر (الجنس سكارم .*Saccharum* spp) ضد مرض التفحم السوطى فى قصب السكر الذى يسببه الفطر *Ustilago scitaminea* ، واستخدمت الاصناف التجارية CO 6806 و C997 وCO 527 كشواهد . أجريت التجارب الحقلية فى موسميين متتاليين (07/2006 و08/2007) . احدثت عدوى صناعية بحقن فطر مرض التفحم السوطى فى عقل الطرز الوراثية المختبرة بطريقتين (أ) طريقة إحداث الثقب التايوانية Taiwanese pin–prick method (TPPM) و (ب) طريقة الغمر Dipping method (DM). حللت البيانات بطريقة رابطة العناقيد الكاملة الموزونة. أظهرت النتائج ان الطرز الوراثية المختبرة السبعة عشر يمكن ان توضع فى أربعة عناقيد (مجموعات) عند إستخدام طريقة الغمر وخمسة عناقيد (مجموعات) عند إستخدام طريقة الثقب التايوانية. عموما ازدادت صفة المقاومة تدريجيا من المجموعة الأولى إلى المجموعة الرابعه ومن المجموعة الأولى إلى المجموعة الخامسه فى طريقتى الغمر وإحداث الثقب التايوانية على التوالى . صنفت 10 طرز وراثية على أنها عالية المقاومة او ذات مقاومة شبيهة للطرازين الوراثيين CO 6806 وCO 997 المستخدمين كشواهد، وبالتالى يمكن استخدامها فى الإنتاج الحقلى التجارى. حاز الطرازان الوراثيان D 9227 وFR 9973 على الدرجة الخامسه (S5) وهى تعادل صفة متوسط المقاومة لمرض التفحم السوطى وبالتالى فهما غير مناسبين للإنتاج التجارى. صنف الطرازان الوراثيان FR 99204 وFR 99314 بين مقاوم وعالي المقاومة، وبالتالى يمكن إستخدامهما للإنتاج الحقلى التجارى .
عموما أثبتت نتائج التحليل الاحصائى ان عشرة طرزًا وراثية صنفت كطرز مقاومة لمرض التفحم السوطى ودرجت فى العناقيد (المجموعات)

3 و4 فى طريقة الغمر والعناقيد 3 و4 و5 فى طريقة احداث الثقب التايوانية، وبالتالى تكون هذه الطرز الوراثية مناسبة للانتاج التجارى .