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The 8-6-6 –Months Protocol: A Quick Method for Field Screening of Sugarcane to Smut

Philip W. Marchelo-d'Raga and Khalid A. Bukhari

Sugarcane Research Centre, Guneid, P.O. Box 511 Khartoum, Sudan

Abstract: This field study was conducted at the Sugarcane Research Centre at Guneid (latitude 15^0N, longitude 33^0E) for two consecutive seasons (2007/08 and 2008/09). The objective was to evaluate and validate the suitability of a new improved field evaluation method (IFEM) in comparison to the conventional field evaluation method (CFEM) which is widely used by pathologists worldwide in identifying resistant sugarcane genotypes to the smut disease. Nine sugarcane genotypes were tested, and sugarcane varieties CO 527, CO 997 and CO 6806 were used as checks. These experimental materials were inoculated artificially by two methods viz: i) Dip method (DM) and ii) Taiwanese pin-prick method (TPPM). Smut resistance evaluations by the new improved 8-6-6 field evaluation method, which took 20 months, identified 10 genotypes as highly resistant (HR) or resistant (R) using TPPM and 12 genotypes as HR or R using DM. The conventional 12-12-12 method (CFEM), which requires 36 months to complete, identified 9 genotypes as HR or R using TPPM and 10 genotypes as HR or R using DM. Differences between CFEM and IFEM were not significant in both DM and TPPM. Therefore, IFEM can be used confidently for routine evaluations of sugarcane germplasm as an alternative to CFEM, to efficiently save time and land.

Key words: Sugarcane smut; artificial inoculation; quick method

INTRODUCTION

The smut (*Ustilago scitaminea* Syd.) disease of sugarcane (hybrids of *Saccharum* species) was first reported from Natal in South Africa (McMartin 1945; Antoine 1961). The disease now occurs in all sugarcane growing countries of the world except Papua New Guinea and Fiji islands (including Irian Jaya). Smut disease is known to severely affect the yield and quality of infected plants (James 1973; Gillaspie and Mock 1983).

Nasr and Ahmed (1974) and Nasr (1977) documented the disease in the Sudan and reported severe smut epidemics in the early sixties that prompted the removal of some excellent sugarcane varieties; namely, NCO 310 and CO 527 from production. Control of the disease, then, relied heavily on chemical seed cane treatment, rouging and destruction of infected plants. These field practices effectively maintained the disease below economic threshold levels. However, these operations were tedious, labour intensive and expensive; thus, making sugarcane smut disease a time consuming and, probably, the most costly disease to control.

Comstock *et al*. (1983) and Burner *et al.* (1993) stipulated that breeding for, and selection of, resistant varieties is the only cheap and cost-effective, viable method of control. The strong genetic control of resistance suggests that progress can be made in developing resistant cultivars (Waller 1970; Walker 1980; Raboin *et al.* 2006). However, the current selection methods for resistance to the disease are painstaking, lengthy and more complicated by reliability on artificial inoculation methods, which are usually modified by an uncertain environmental component for pathologists (Waller 1970; James 1972). Also, the shy flowering of some sugarcane genotypes and problems associated with pollen viability in some geographical zones is another constraint for breeders. Nevertheless, these would be made more cost-effective if susceptible varieties could be pinpointed and eliminated much sooner in the screening programme than current methods permit. Furthermore, Raboin *et al.* (2006) cautioned that while breeding for smut resistance is efficient, it requires complicated screenings.

The current conventional field evaluation method (CFEM) needs 36 months to effectively complete the three crop cycles, required, to evaluate and judge a sugarcane genotype as either resistant or susceptible to the smut disease. Tentatively, CFEM requires 12 months for plant cane (PC), 12 months for first ratoon (R1) and another 12 months for the second ratoon (R2) or 12-12-12), before a final assignment of resistance reaction types could be made. The present study was conducted with the objective of verifying / validating an improved field evaluation method (IFEM) that took a shorter time of 20 months, i.e. 8 months for PC, 6 months for R1

and another 6 months for R2, or 8-6-6. Subsequently, should IFEM prove to be effective and gives results comparable to that of CFEM, it is envisaged that it will effectively increase and boost the output of resistant sugarcane genotypes to the disease.

MATERIALS AND METHODS

This study was conducted for two consecutive seasons (2007/08 and 2008/09) in the Experimental Farm of the Sugarcane Research Centre at Guneid (latitude 15^{o}N, longitude 33^{o}E). The soil at the experimental site is vertisols (about 64% clay, 0.09% N and 2-8 ppm available P) with low permeability and alkaline in reaction (pH=8.2). The climate of the locality is tropical with low relative humidity.

Planting materials and land preparation

Standard methods of cane seed bed preparation of heavy disking, harrowing and ridging at 1.5 m row spacing were adopted. Nine sugarcane genotypes; namely, B 70531, B 79136, BJ 7451, BJ 7938, BJ 82105, BT 74209, COC 671, DB 75159 and TUC 75-3 were evaluated using the three commercial varieties (CO 527, CO 997 and CO 6806) as checks. Single budded (eyed) cane seed pieces or setts were prepared from 8-10 month old field grown cane crop of each genotype or variety. The setts were artificially inoculated by fresh smut teliospores collected from the variety NCO 376 by the standard methods of (i) Taiwanese pin-prick method (TPPM) and (ii) dip method (DM).

Taiwanese pin-prick method (TPPM)

Two pin punchers were made at the base of each bud, on each sett after the pin was dipped into a freshly prepared spore paste 1-1.5 g spores/10 ml water (equiv. 1x10^{5} to 1x10^{6} spores ml^{-1}) (Bock 1964). The inoculated setts were incubated at room temperature, in the laboratory, under moist conditions for 24 hrs to enhance spore germination before being planted in the field. The plot size was 1 row of 5 m length. Twenty single budded (eyed) cane setts were planted in each plot as double setts. The trial was laid in a randomized complete block (RCB) design with three replications.

Dip method (DM)
The field layout, plot size and number of setts per plot were as in TPPM. However, the cane setts were inoculated by dipping in a spore suspension at a concentration of 1g spores/litre of water (equiv. $1x10^7$ spores ml^{-1}) for 15-20 minutes. Thereafter, the setts were also maintained under plastic bags and planted in the field after 24 hours incubation as above. The trial was also laid in RCB design with three replications.

Data collection and evaluation of resistance
For both DM and TPPM trials, data were collected on disease incidence parameters, i.e. number of whips and number of diseased and healthy stools from which disease incidence was determined. Counts started at first whip emergence (60-90 days after planting) and continued at bi-weekly intervals for 8 months for PC and 6 months for each of R1 and R2. Resistance reaction types were then derived by rating the percentage of smut infection on the standardized rating scale of Satya Vir Beniwal (1978). This was done in a similar manner as previously administered to the 12-12-12 months CFEM method, using the same sugarcane test genotypes which were also inoculated by both DM and TPPM (Marchelo *et al.* 2008).

Statistical analysis: The results obtained by IFEM methods were then compared with those of CFEM. Thereafter, it was validated statistically by the t-test using the statistical software MSTATC. Data were transformed to square root prior to analysis.

RESULTS AND DISCUSSION

The evaluation of sugarcane genotypes by CFEM identified 9 genotypes as HR or R for TPPM and 10 genotypes as HR or R for DM. On the other hand, IFEM identified 10 genotypes as HR or R for TPPM and 12 genotypes as HR and R for DM (Table 1). The actual number of genotypes in each reaction type group and their corresponding percentages are given in Table 2. Usually, some differences under field conditions are not uncommon. The performance of, some of these genotypes and inoculation methods have previously been elucidated under

the Sudan environment (Marchelo *et al.* 2008; Marchelo and Bukhari 2009). Also, James (1972) indicated that under field conditions some fluctuations, due to the largely uncertain environmental factors, should be contended with. However, Nallathambi *et al*. (1998) suggested that the use of dikaryotic mycelium in hypodermic inoculations of cane shoots by syringe injection completely eliminated disease escape.

Nonetheless, it should be noted that these differences, validated by t-test, were not significant for CFEM and IFEM evaluation protocols, in both DM (t=0.82) and TPPM (t=0.88). Therefore, the new 8-6-6 IFEM protocol (=20 months duration), which is shorter by 16 months than CFEM, can thus be used as an alternative method instead of the 12-12-12 CFEM protocol (=36 months duration) in routine field evaluation of sugarcane germplasm as it is efficient in the use of both time and land. Likewise, Nallathambi *et al.* (1998), working on a similar scenario in India to avoid the time consuming and resource intensive field evaluation methods currently in use there, reported that alternative-evaluation by a histological staining technique was effective under Indian conditions. They further stressed that this technique was more rapid, precise, suitable for large number of samples and economical. However, its successful use requires a suitably above average laboratory and a trained eye to equate fungal mycelium growth in the internal cane tissues under test to their corresponding reaction types. Also, Gillaspie and Mock (1983), working on similar evaluations in a containment greenhouse tests, indicated that 7-8 months was sufficient. They, however, used pre-sprouted sugarcane setts shoots of which were then inoculated by teliospore suspensions when they were 8-12 cm tall by hypodermic syringe injection. This, in our view, sufficiently eliminated disease escape and achieved actual physiological resistance. However, it is more labour intensive and taps on meagre resources and is thus, not suitable for large number of samples. Also, testing sugarcane genotypes only at the PC cycle will tend to give a higher yield of resistant varieties than is actual, since bud scale resistance is not tested by this method. The implication of this is that materials tested here as resistant could later succumb to infection under field conditions through bud infections by wind-borne spores in standing cane.

Table 1. Comparative percentage of smut infection and reaction types of sugarcane varieties in the second ratoon crop cycle for the two field evaluation and inoculation methods

Geno-type	DM				TPPM			
	PSI	NOW/F (x1000)	RAT	RTP	PSI	NOW/F (x1000)	RAT	RTP
The '12-12-12' conventional field evaluation method, CFEM (2002/03-04/05)								
B 70531	01.03	0.01	1	HR	05.80	0.01	2	R
B 79136	05.94	0.75	2	R	04.70	0.75	2	R
BJ 7451	15.15	1.62	5	MS	22.50	1.62	5	MS
BJ 7938	17.92	0.15	5	MS	44.80	0.15	7	S
BJ 82105	03.03	0.12	1	HR	02.90	0.12	1	HR
BT 74209	06.37	1.04	2	R	09.70	1.04	4	R
COC 671	12.88	0.61	4	R	04.70	0.61	2	R
DB 75159	00.67	0.10	1	HR	01.70	0.10	1	HR
TUC 75-3	01.48	0.08	1	HR	00.40	0.08	1	HR
CO 527	07.49	0.50	3	R	18.90	0.50	5	MS
CO 997	02.39	0.19	1	HR	03.20	0.19	1	HR
CO 6806	00.42	0.05	1	HR	08.90	0.05	3	R

Table 1. Cont.

Geno-type	DM PSI	DM NOW/F (x1000)	DM RAT	DM RTP	TPPM PSI	TPPM NOW/F (x1000)	TPPM RTP	TPPM RTP
The '8-6-6' improved field evaluation method, IFEM (2007/08-08/09)								
B 70531	0.71	0.15	1	HR	02.00	00.48	1	HR
B 79136	3.33	0.79	1	HR	24.46	03.71	5	MS
BJ 7451	4.67	2.34	2	R	21.94	03.00	5	MS
BJ 7938	5.61	0.87	2	R	05.99	03.08	2	R
BJ 82105	0.00	0.00	1	HR	00.00	00.00	1	HR
BT 74209	1.50	0.22	1	HR	01.80	00.78	1	HR
COC 671	2.91	0.65	1	HR	05.35	01.76	2	R
DB 75159	0.33	0.02	1	HR	02.29	20.60	1	HR
TUC 75-3	1.32	0.26	1	HR	02.22	00.87	1	HR
CO 527	2.49	0.55	1	HR	10.50	04.18	4	R
CO 997	2.03	0.11	1	HR	03.24	00.53	1	HR
CO 6806	0.31	0.12	1	HR	02.29	00.34	1	HR

DM= dip inoculation method; TPPM= Taiwanese pin-prick method; PSI= percentage smut infection on stool basis; NOW/F= number of whips per feddan; RAT= rating; RTP= reaction type; HR= highly resistant; R=resistant; MS= medium susceptible; S= susceptible

Table 2. A summary of the reaction types and percentages of smut infection evaluated by the conventional CFEM and improved IFEM field methods

FEM	Number and percentages of genotypes showing different reactions					
	HR	R	MS	S	HS	Total
			Inoculation by TPPM			
CFEM	4 (33.3)	5 (40.2)	2 (16.7)	1 (08.3)	0 (00.0)	12
IFEM	7 (58.3)	3 (25.0)	2 (16.7)	0 (00.0)	0 (00.0)	12
			Inoculation by DM			
CFEM	6 (50.0)	4 (33.3)	2 (16.7)	0 (00.0)	0 (00.0)	12
IFEM	10(83.3)	2 (16.7)	0 (00.0)	0 (00.0)	0 (00.0)	12

HR = highly resistant; R= resistant; MS = medium susceptible; S = susceptible; HS = highly susceptible; FEM= field evaluation methods; CFEM = conventional field evaluation method; IFEM = improved field evaluation method; Figures in parenthesis are the percentage of genotypes in each category of reaction type

CONCLUSION

The time needed for IFEM is 20 months which is about 16 months shorter than that for CFEM. Therefore, it can be adopted in the routine screening of sugarcane genotypes for resistance to the smut disease as an alternative to CFEM for efficient use of time and land.

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بروتكول 8-6-6 :اختبار حقلى سريع لمسح مرض التفحم فى قصب السكر

فليب وانى مارسيلو دراقا وخالد إبراهيم بخارى .

مركز بحوث قصب السكر بالجنيد - شركة السكر السودانية
ص.ب 511 الخرطوم - السودان

المستخلص : اجريت هذه الدراسه بمركز بحوث قصب السكر بالجنيد (خط عرض 15^0 شمال وخط طول 33^0 شرق) لموسمين متتالين (2008/2007 و2009/2008) بهدف تقييم صلاحية اختبارحقلى محسن لمرض التفحم، مقارنة مع الطريقة التقليدية والتى تستعمل عالميا للتعرف على الاصناف المقاومة لمرض التفحم فى محصول قصب السكر. اختبرت تسعه طرز وراثية من محصول قصب السكر، مقارنة بثلاثة اصناف (CO 527، CO 997 و CO 6806) كشواهد حقنت الطرز الوراثية اصطناعيا بطريقتين (i) الغمر (Dip method)و(ii) طريقة حداث الثقب التايوانية (Taiwanese pin–prick method). جرى تقييم لمقاومة المرض باستعمال طريقة 8-6-6 خلال 20 شهرا حيث تم التعرف بطريقة الحقن على عشرة طرز وراثية عالية المقاومة أ ومقاومه وبطريقة الغمرعلى أثنى عشر طرازاً وراثياً عالى المقاومة أو مقاوم. تم التعرف بالطريقة التقليدية (12-12-12)، والتى تحتاج الى 36 شهرا، على 9 طرز وراثية عالية المقاومة أو مقاومة باستخدام طريقة الحقن و10 طرز وراثية عالية المقاومة أو مقاومة بطريقة الغمر . أثبتت الدراسة أن الفروقات بين الطريقتين 12-12-12 و8-6-6 غير معنوية وذلك باستعمال طريقتى الحقن والغمر، لذلك يمكن استخدام الطريقة المحسنة (8-6-6) لزيادة الكفاءة من حيث الزمن والمساحة بديلا للطريقة التقليدية (12-12-12).