

RESEARCH ARTICLE**ASSESSMENT OF POLLEN FERTILITY, CANE YIELD AND ETHANOL CONTENT IN SUGARCANE PROGENIES DEVELOPED BY THE MODIFIED POLYCROSS METHOD****G. Olaoye¹, J.O. Olaoye², F. O. Takim¹, A.M. Idris¹ and F. Bankole¹****Abstract**

Performance of breeding lines in advanced yield trials is prerequisite to identification of superior genotypes intended as replacement to the existing cultivars. To this end, 10 advanced sugarcane lines from the Unilorin Sugar Research Institute (USRI) breeding programme were assessed for their flowering behaviour, sugar (cane yield and sucrose content) and ethanol yields using a randomized complete block design with four replications during 2011-2013 cropping seasons at the institute's research farm. The incidence of the disease in commercial fields reached upto 100% in susceptible cultivars (Rassaby et al. 2004). The results showed that all the progenies were highly fertile and could be utilized as male parent in crosses. On the basis of pollen morphology, the genotypes were classified as either Sulcate or Colpate. Many of the progenies yielded significantly ($P < 0.001$) higher yield than a few check varieties and progeny USRI/08/63 recorded the highest cane yield comparable to the yield of the best standard variety Co 6806. Among the progenies, the highest ethanol yield was obtained from progeny USRI/08/03 (15% ethanol) followed by four progenies (USRI/08/16, USRI/08/63, USRI/08/68 and USRI/08/85) with 10% ethanol. Since different genotypes were identified combining high cane and ethanol yields, the results highlighted the advantage of designing a separate breeding programme for the development of high ethanol content sugarcane varieties.

Key words: *Saccharum officinarum*, pollen morphology, sugar yields, ethanol content, polycross

Introduction

Sugarcane (*Saccharum officinarum* L.) breeders routinely carry out hybridization procedures which normally involve the use of highly fertile sugarcane genotypes as source of pollen (males) in crosses with male sterile types (as females) followed by raising of the fuzz (true sugarcane seeds). Although the degree of anther dehiscence when viewed with the hand lens gives an indication of sexuality of a flowering variety, classification based on microscopic examination of the pollen grains is a more realistic procedure for correctly classifying flowering sugarcane varieties either as male or female.

The occurrence of flowering under field conditions is variable and is also influenced by variety as well as prevailing environmental conditions in a locality. Previous studies have shown that flowering is a complex process consisting of multiple stages of development with each stage having specific environmental and physiological requirements. The environmental conditions may include a combination of factors such as diurnal temperatures, specific day length, elevation, temperature and moisture requirements (Van-Breeman et al. 1962; Clements and Awada 1967; Coleman 1963; Gosnell 1973; Moore 1987; Moore and Nuss 1987; Araldi et al.

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2010), rainfall amount and distribution (Olaoye 1996), sub-optimal photoperiod (Nayamuth et al. 2003; Berding 2005), rising atmospheric concentrations of CO₂ (Rosenzweig et al. 1995) and pollution levels.

Furthermore, as the world demand for alternative source of fuel increases, attention has been focused on non-fossil source of fuels which include crops such as sugarcane, cassava (*Manihot utilissima*), jatropha (*Jatropha cactus*) among others. According to Deepland (2005), sugarcane is one of the plants having the highest bioconversion efficiency of captured sunlight through photosynthesis to fix around 55 tonnes of dry matter/ha of land on an annually renewable basis. For example, the crop has been used in Mauritius as a part of energy conservation and efficiency measures to minimize cogenerated energy (steam and electricity) utilized in cane processing and also export excess electricity to the grid. Similarly, Brazil has diversified sugarcane breeding efforts to include development of varieties for ethanol (biofuel) generation from the crop, as source of fuel for their automobile by transforming sugarcane into about 12 x 10⁹ litres, 1/5 of which is in anhydrous form (Gonzalez and Galvez, 1998). A corollary is that sugarcane breeding efforts in other sugar producing countries have been diversified into development of varieties for specific end uses such as high sugar, high fibre and ethanol content sugarcane varieties.

Secure, reliable and affordable energy supplies are fundamental to global economic stability and growth. The challenges of sustainable development are great and the importance of energy in achieving sustainable development is predicated upon the search for sustainable programmes for generation of energy from biomass. Access to affordable energy services is fundamental to human activities, development and economic growth. Biomass is considered to be one

of the key renewable resources of the future at both small and large scale levels. The development of biomass as a source of clean and renewable energy has been encouraged because of its benefits especially environmental sustainability (Keeney and DeLuca 1992). Olaoye (2011) reported that production of biofuels has the beneficial effect in increasing a sustainable fuel supply for the future. The activities through the production chains of biofuels provide jobs and socio-economic development in rural areas. The use of ethanol as fuel is capable of reducing the adverse foreign trade balance. Colmac (2009), Van Gerpen et al. (2007) and Olaoye (2011) noted that the cost benefit ratio of production of biofuels may be higher compared to fossil fuel but biofuel does not contribute to greenhouse effect problem which is a major problem with other known energy sources.

Since the inception of sugarcane varietal development activities in Nigeria, breeding efforts have concentrated on the development of high yielding (cane yield and sucrose content) varieties without diversifying breeding efforts to the development of varieties for specific end-uses such as high fibre (for coenergy generation) or high ethanol (biofuel) content sugarcane varieties. Although previous studies (Oworu 1987; Fadayomi et al. 1995), have shown that flowering is not a desirable trait in sugarcane because of the diversion of photo-assimilates into flowering and seed production to the detriment of sucrose accumulation, it is required in sugarcane breeding for varietal development. Consequent upon our interest to diversify breeding efforts into the development of improved sugarcane varieties for other specific end uses other than manufacturing of refined sugar, 10 of the 97 progenies from our 2007 modified polycross scheme which combined high cane yield with high sucrose in the juice at the preliminary yield testing stage (which are also the flowering type), were

selected for further yield evaluation in the savanna ecologies. In this part of the study, the performance of the progenies for cane yield, its related traits and ethanol content under large plot sizes were investigated. The fertility status of the flowering types was also determined with the view to classify them either as male or female for effective utilization in hybridization programmes.

Materials and methods

Source of genetic material

The genetic material used comprised 10 flowering sugarcane clones which were selected from among 97 progenies evaluated for their yield potential and other attributes at the research farm of the Unilorin Sugar Research Institute (USRI), Ilorin in 2009 (Olaoye et al. 2010). These progenies were the products of the 2007 modified polycross breeding scheme, which was developed in the institute to implement planned crosses overcoming the lacunae of specialized glasshouse and stock solution for effective hybridization under a controlled condition to prevent contamination from unwanted pollen source. The details of the scheme have been described in an earlier paper (Olaoye 2001).

Field experimentation

The study was conducted during 2011/2012 and 2012/2013 growing seasons at the USRI, farm, Ilorin in the Southern Guinea Savanna (SGS) agro-ecological zone of Nigeria (Lat 8° 29 and Long 4° 35E). The rainfall pattern is usually bimodal with its highest peak in July and September and a break between mid-July and late August. The average annual precipitation of the area is 1250 – 1500mm with temperature ranging between 19°C and 33°C. The 10 sugarcane progenies were evaluated along with five commercial varieties as checks. The experimental design was a randomized complete

block with three replicates. The trials were laid out in four row plots, 5 meters long with 1.5m between the plots and an alley of 1m between plots. Three-budded sugarcane setts used as planting materials were laid horizontally in the furrows and eight setts were planted per row. All agronomic practices including weed control and fertilizer application were carried out according to the standard practices.

Data collection

Cane yield and juice analyses

Data were recorded in five random stools selected from the two middle rows on yield parameters *viz.* tiller count, stalks/stool, stalk length, stalk diameter, millable cane population, internodes/stalk and length of internode. Data were also collected at harvest on cane yield, single stalk weight and sucrose accumulation in the juice. For cane yield, all millable cane stalks from the two middle rows were harvested and weighed on scale and recorded in kilograms (kg). The weights were later converted into cane yield in tonnes per hectare. Single stalk weight was measured as the weight of three randomly selected single cane stalks per plot and recorded in kg. Measurements of Brix, which is the percentage by weight of the soluble solids in the juice when squeezed from a mature or crushed sugarcane stalk with an extractor and measured and read on a refractometer were collected from three randomly selected millable stalks in a plot.

Pollen characteristics

Arrows were collected from three stalks/plot and taken to the laboratory of the Department of Plant Biology for microscopic examination of the pollen grains. Matured anthers on the spikelets at shedding period were collected in sample bags and examined for pollen morphology and viability tests under a light microscope. The anthers were squashed on the

microscope slide to remove the pollen grain from the anthers. Few drops of stain lacto-phenol (cotton blue) were added and covered with cover slip to prevent the pollen grains from displacement. The prepared slides were then examined under the light microscope. Viable (fertile) pollen grains appeared large, fully round and dark in colour while inviable (infertile) pollen grains appeared clear, empty and colourless. The pollen grains were then counted and sorted into fertile and infertile pollen grains and the number of fertile pollen was expressed as percentage over total number of pollen grains. Based on the percentages of fertile and infertile pollen grains, the genotypes were classified into males and females. Genotypes with high percentages (50 – 100%) were rated as males and genotypes with lower percentages (0-49) were rated as females.

Photomicrograph

The structure and characteristics of pollen grains were determined using the photomicrographic scanning machine in the Department of Plant Biology, University of Ilorin. On the basis of structure and characteristics, the pollen grains were characterized as either inviable or viable. The viable pollen grains were further grouped as either colpate (elongated aperture or furrow) or sulcate (many pores).

Determination of ethanol (biofuel) content

The biofuel component comprising sugarcane juice extractor, fermenter and distillation unit were designed and constructed in the Department of Agricultural and Biosystems Engineering of the University of Ilorin (Fig. 1). Three litres of sugarcane juice was extracted from each of the progenies with the aid of the juice extractor. This was followed by the addition of 1.2 g of commercial Baker's yeast, dry *Saccharomyces cerevisiae* to each of the three samples of sugarcane juice per progeny. The

concentrate was fermented for a period of 48 h in the fermenter with stirring under anaerobic conditions. The juice was later distilled and bioethanol yield determined for each genotype using the hydrometer values. Brix was determined with the hand refractometer while a viscometer was used to determine the Kinematic Viscosity of the slurry after distillation at 27°C. The procedure was repeated three times for each genotype. Other data collected included time of grating, weights of grated canes, volume of juice, brix value and machine loss. Amount of juice yield was later calculated using the formula:

$$\text{Juice yield (\%)} = [J_e / (J_e + W_r)] \times 100$$

where J_e = weight of extracted juice, W_r = weight of residue.



Fig. 1. View of sugarcane juice extractor (left), fermenter (middle) and distillation unit (right)

Results and discussion

Means with standard errors attached (SE_{\pm}), ranges in the means and coefficient of variation ($CV\%$) for pollen characteristics in 10 USRI progenies and four check varieties are presented in Table 1. The results showed that the number of viable pollen grains was quite higher than the inviable pollen grains with a difference of greater than 95%. The range in the means for the viable pollen grains among the genotypes, which is an indication of differences

Table 1. Means with standard errors (SE±) for pollen fertility in 10 sugarcane progenies and four check varieties

Trait	Mean±SE	Min	Max	Range	CV(%)
Total pollen count	582±174	88	1104	1016	53.0
No. of viable pollen grains	523±173	65	1026	961	63.4
No. of inviable pollen grains	26.6±8.52	8.7	44	35.3	12.9
% Viability	96.72±3.48	88.07	96.06	7.99	5.4

among them for this trait, as well as CV were large compared to the values obtained in respect of inviable pollen grains.

Fig. 2 shows the different pollen characteristics encountered among the flowering genotypes. The inviable pollen grains (top) had clear, collapsible and colourless morphology which failed to absorb the stain. This feature was observed in USRI/08/58 which had the highest number of inviable pollen grains relative to total pollen count. Viable pollen grains appeared round and took the blue colour of the stain (lactophenol or cotton blue) and majority of the USRI progenies are in this category as they exhibited high percentage of stained pollen grains and therefore were characterized as viable. The viable pollen grains were further classified as either *sulcate* (middle) or *colpate* (bottom). The *sulcate* type were characterised by possession of numerous pores in ring when viewed under light microscope while the *colpate* type is characterised by the

presence of two apertures in their pollen after viability test is carried out. Three of the progenies (USRI/08/03, USRI/08/43 and USRI/08/85) as well as the three standard varieties (ILS-001, ILS-002 and Co 6806) were classified as *colpate* because they had aperture in their viable pollen grain, while the others could be classified as *sulcate* as they possessed many pores in viable pollen grains.

The mean values for pollen characteristics among the USRI progenies and check varieties are presented in Table 2. Many of the progenies have higher percentage of viable pollen than the check varieties with USRI08/80 having the highest pollen viability (98.84%) and USRI/08/43 having the lowest percentage (55.16%) viable pollen. Based on pollen viability and pollen fertility, flowering genotypes were classified as either female (0-49%) or male ($\geq 50\%$). Since all the test genotypes had high pollen fertility, they were classified as male fertile which formed the source of pollen in our hybridization programme.

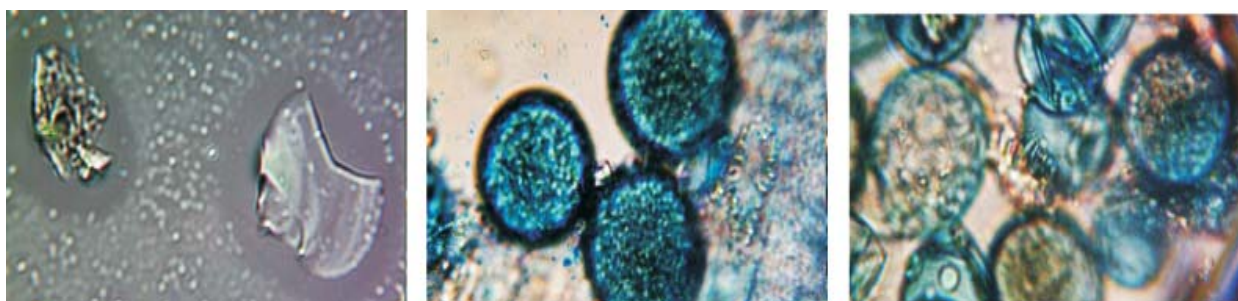


Fig. 2. Different types of pollen morphology in sugarcane genotypes: inviable pollen grains (left), *sulcate* viable pollen grains (middle) and *colpate* viable pollen grains (right).

However, many of the genotypes with high pollen count also had high % pollen fertility which is contrary to earlier reports (Olaoye 1996) indicating inverse relationship between pollen production and fertility. A few progenies used in this study as well var. ILS-002 were non-flowering types, either at the time of selection (progenies) or release (ILS-002). However, results from this study showed that they flowered (and some of them profusely) and this shift is mainly attributed to climate change effects as supported by recent observations from our yield testing programme (Olaoye et al. 2010) that extent of flowering behaviour, as well as

sexuality (male or female) in sugarcane do change relative to changes in climatic factors (Badalou, pers. comm.).

Cane yield and associated traits in the progenies and check varieties are presented in Table 3. There were significant differences among the genotypes for almost all the traits except stalk length and stalk diameter. Single stalk weight and millable cane population jointly contributed to overall cane tonnage as the high yielding genotypes (as in USRI/08/03, Co 6806, USRI/08/63 and USRI/08/46) showed superiority for these two traits. Low yielding

Table 2. Pollen characteristics and sexuality of 10 sugarcane progenies and two check varieties

S.No.	Genotype	Total pollen count	No. of viable pollen	No. of inviable pollen	% Viability	Sexuality
1.	USRI/08/03	727	692	34.7	95.18	Male
2.	USRI/08/16	392	374	17.3	95.41	Male
3.	USRI/08/43	1104	609	35.0	55.16	Male
4.	USRI/08/46	826	788	37.7	73.86	Male
5.	USRI/08/58	88	65	23.3	98.65	Male
6.	USRI/08/63	592	584	8.7	92.84	Male
7.	USRI/08/68	489	454	35.7	93.10	Male
8.	USRI/08/80	449	418	31.7	98.84	Male
9.	USRI/08/85	1038	1026	16.0	95.45	Male
10.	USRI/08/87	462	441	21.0	98.04	Male
11.	CO6806	614	602	12.3	95.70	Male
12.	ILS-002	396	379	16.7	94.55	Male
13.	ILS-001	808	764	44.0	74.10	Male
14.	Local check	162	123	39.0	75.92	Male
	SED	246.5	245.4	12.14	4.93	
	LSD(0.05)	506.6	504.4	Ns	10.14	
	F-Test	**	**	Ns	**	
	%CV	51.9	57.5	55.8	6.7	

Table 3. Mean cane yield and associated traits in 10 sugarcane progenies and five check varieties

Genotype	Stalks/ stool (no.)	Tiller count (no.)	Stalk length (m)	Stalk diameter (cm)	Internode length (cm)	Internode stalk (no.)	Millable canes (no.)	Single stalk weight (kg)	°Brix	Cane Yield (t/ha ⁻¹)
USR108/03	30	167	1.50	1.93	8.65	12	74	0.70	20.3	68.9
USR108/16	23	110	1.11	2.07	6.80	11	47	0.50	19.6	58.4
USR108/43	9	83	1.34	2.20	9.59	10	39	0.60	20.1	58.6
USR108/46	34	141	1.52	2.40	8.67	13	53	0.83	20.4	67.5
USR108/58	10	73	1.38	2.53	7.65	14	25	0.80	20.0	56.1
USR108/63	24	128	1.23	1.93	8.73	14	52	0.70	20.0	71.6
USR108/68	9	85	1.05	2.38	8.52	13	36	0.77	19.7	62.8
USR108/80	21	126	1.58	2.10	9.18	15	46	0.67	20.4	62.5
USR108/85	6	74	1.03	2.03	9.54	9	32	0.33	19.7	55.4
USR108/87	15	120	1.17	1.97	8.99	12	45	0.40	19.9	63.0
Co 6806	24	60	1.36	2.57	8.70	16	52	0.97	20.4	71.0
Co 957	10	68	1.15	2.15	8.54	16	38	0.47	20.9	57.6
ILS-001	12	70	1.23	2.97	9.40	11	16	0.50	19.7	54.4
ILS-002	18	94	1.17	2.13	7.79	14	43	0.50	19.6	60.4
Local check	25	158	1.10	1.97	8.64	11	61	0.40	20.0	8.14
Mean	18	104	1.34	2.22	8.63	12.73	43.93	0.61	20.05	61.09
SED	4.64	27.16	0.23	0.28	0.64	1.79	23.26	0.269	0.69	9.24

genotypes on the other hand (Co 957, Local check and USRI/08/43) had high brix content which also supports earlier findings (Smith and James 1969; Miller and James 1971) of an inverse relationship between cane yield and sucrose in the juice. All the progenies were significantly higher than the check variety for cane yield. Progeny USRI/08/63 had the highest cane tonnage which was comparable to the yield of the best standard variety (Co 6806). Two other progenies (USRI/08/03 and USRI/08/46) also combined high cane yield with acceptable brix content. The difference in cane yield between the highest yielding genotypes and progeny with the check variety was 23t/ha representing a yield advantage of 32.77%.

The ethanol yields in the progenies and those of three standard varieties are presented in Table 4. Among the progenies, the highest ethanol yield was obtained from progeny USRI/08/03 with 15% ethanol followed by progenies USRI/08/16, USRI/08/68 and USRI/08/80 with 10% ethanol and the lowest value of 5% was recorded in progenies USRI/08/46, USRI/08/638 and USRI/08/85 after 48 h of fermentation. The slurry from progeny USRI/08/80 had the highest kinematic viscosity of 8.5 cm³/s while USRI/08/68 had the least of 1.0 cm³/s. The ethanol yield obtained in respect of the progenies was low when compared to values obtained in the standard varieties. This may be due to the fact that the determinations in 2011 were carried out as soon as

Table 4. Mean ethanol content in sugarcane progenies and two check varieties

Genotype	Brix value before fermentation	pH value		Volume of distillate (ml)	Ethanol (%)	Kinematic viscosity of the slurry (cm ³ /s) at 27°C
		Before fermentation	After fermentation			
USRI/08/03	18.5	5.0	3.6	470	15	6.1
USRI/08/16	18.5	5.0	3.5	420	10	5.4
USRI/08/43	18.5	5.2	3.4	815	8	1.1
USRI/08/46	14.0	5.3	4.3	1060	5	1.2
USRI/08/63	14.5	5.4	3.4	925	5	4.5
USRI/08/68	16.0	4.8	3.6	500	10	1.0
USRI/08/80	15.0	5.2	3.5	750	10	8.5
USRI/08/85	16.5	4.8	3.5	680	5	5.3
USRI/08/87	15.0	5.0	3.5	650	8	2.5
USRI/08/58	14.0	4.8	3.6	410	8	2.3
Mean						
ILS-001 ⁺	18.0	4.7	4.3	1390	51	2.1
ILS-002 ⁺	18.0	4.8	4.1	1460	46	5.0
Co 957 ⁺	18.0	4.8	3.5	3,200	26	6.1

⁺ Determination made in 2011 season

flowering commenced while the activity in 2012 was carried out long after flowering process was completed.

The results from the present study revealed that different genotypes exhibited superiority of performance especially with respect to sugar and ethanol yields. This implies that development of high ethanol content sugarcane varieties is also feasible using the current genetic resources at our disposal. Furthermore, since these progenies are highly fertile, they can serve as source of genes for the development of high sugar and ethanol content sugarcane varieties.

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