GENOTYPIC RELATIONSHIPS AMONG FLOWERING BEHAVIOUR, POLLEN FERTILITY, CANEYIELD AND SUCROSE ACCUMULATION IN SUGARCANE GERMPLASM ACCESSIONS IN SAVANNA ECOLOGY OF NIGERIA

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Abstract

Assessment of Germplasm materials is a prerequisite for their utilization either as parents or cultivars in hybridization programme in Nigeria aimed at the development of future varieties. This study was therefore conducted to determined the relationship among flowering behaviour, pollen fertility, caneyield and sucrose accumulation in sugarcane germplasm accessions in a savanna ecology of Nigeria. The study was conducted at Teaching and Research Farm, Sugar Research Institute, University of Ilorin Kwara State Nigeria for two (2) cropping seasons 2010/2011, latitude $8^0$29N and longitude $4^0$35E. The experimental design used was Randomized Complete Block Design (RCBD) with three (3) replicates. The main objective of the study was to identify clones that will be suitable for hybridization aimed at development of high yielding sugarcane varieties for cultivation on Sugar Estates in the savanna ecology of Nigeria. Results showed that the accessions differed significantly for most of the characters studied. However, few of the accessions were found to high in caneyield which could eventually replace the current low yielding varieties on Sugar Estates in Nigeria.

The ultimate goal in sugarcane (Saccharum offienarum L) breeding is to develop genetically improved varieties with high sugar yield (cane yield and sucrose content) that can be economically sustainable over several ration crops. Therefore, germplasm materials are usually assessed for their breeding behaviour with the objective of utilizing them as parents in hybridization for evolving new and superior progenies intended as replacement of the existing cultivars. This process in sugarcane depends essentially on the flowering behavior (flowering or non flowering) sucrose

accumulation (high and low contents) and the extent of pollen fertility (viable or non viable pollen grains) in flowering sugarcane clones.

Flowering in sugarcane is a complex physiological process which consists of multiple stages of development (initiation, flagging, emergence and pollen shedding). With each stage having specific environmental and physiological requirements Araidi, R.F.M.I, Silva, E.O, & I.D Rodriquz (2010). Environmental factors such as durnal temperatures (Clements and Awada 1967: Adejuwon. 1988). Rainfall amount and distribution (Olaoye, 1996) as well as intermittent occurrences of night temperature below 18^0C during the period of floral induction, which reduces flowering intensity and/or delay seedling emergence (Coleman, 1963: Gosnell 1973: Pereira, A.R.V, Baribiri, & N.I Villanova (1983). Also impact on sugarcane flowering and pollen fertility. Frequent occurrences of daytime temperatures exceeding 31^0C acting singly or in combination with moisture stress. Have also been implicated in similar reduction in flowering intensity or delay seedling emergence Ellis, T.O J.F, Van-Breemen & G. Areeneux (1967): Nuss and Brent 1977).

In the attempt to evolve sugarcane genotypes that will be capable of producing high cane yield over several crop cycles breeding programmes routinely perform several crosses. This hybridization procedure involves the use of highly fertile sugarcane perform genotypes as source of pollen (males) which are then crossed to male sterile types (as females). Followed by raising of the fuzz (true sugarcane seeds). However. Empirical data suggest that flowering in sugarcane (extent and intensity) and sexually (Maleness or femaleness) are usually variable especially at many tropical locations due to sub-optimal photoperiod (Berding and Hurney 2005). A situation which is often compounded by moisture stress with the consequence of poor pollination success from hybridization programmes. The implication is that classification of flowering sugarcane varieties as male or female either on the basis of degree of pollen shed or pollen viability test has to be carried out routinely during the breeding season in order to ensure success in pollination.

Apart from flowering behaviour, sugarcane breeders also evaluate new germplasm accessions in order to identify genotypes that are highly productive over a period of time so that they can serve as replacement to existing cultivars or used in cross combinations to evolve superior genotypes that will serve as future varieties. This study reported herein was therefore undertaken with the objectives to (i) assess flowering behaviour and sexuality of the new germplasm accessions from Berbados (West indies) along with six standard varieties. (ii) determine the relationship among flowering, pollen factly and sugar yields in the accessions and (iii) identify superior genotypes from among the new accessions which could be used either as parents in crosses or evaluated further on the sugar estates as potential varieties.

Materials and Methods

Thirty (30) exotic sugarcane germplasm accessions representing the first batch of the genetic resources from Berbados (West Indies(and six (6) standard varieties (as cheeks) were evaluated at the Research Farm of the Unilorin Sugar Research Institute (USRI). Ilorin, in the southern guinea savanna agro-ecological zone of Nigeria (Lat 8 29^0 and Log 4-35^0). Three of the check varieties, (Co957, B47419& Co62175) are commercial varieties while the remaining three were varieties developed at USRI. Two of which have been released (ILS-001 and ILS-002). The ranfall pattern in the ecology is bimodal with the highest peak in July and September and a break usually between mid-July and late August of every year. The average annual precipitation of the area is 1250 1500mm with temperature ranging between 19^0C and 33^0C.

The accessions were obtained from the Josepdam Sugar Company estate. Bacita. The experimental design was a three-replicate randomized complete block (RCBD). The genetic materials were planted into single-row plots 5m long and 1.6m between the rows with one meter (1m) alley between the plots during 2010/2011 growing season. Three-budded sugarcane sets were laid in furrows at a depth of 15 centimeter (cm) and covered with soil. Pre-mergence herbicide was immediately applied to control weeds. Supplementary weeding was thereafter carried out as necessary throughout the period of the experimentation fertilizer application was carried out as split dosage at the recommended rate of 150kgNha. The first dose was applied immediately after planting while the second dose was applied six months after the initial application. The fields were irrigated between November 2010 through April 2011 to ensure adequate moisture supply throughout the period of dry season and also immediately after harvesting the crops in December 2012. Thereafter, the ratoon crops were continuously irrigated immediately after harvest until May, 2012 when the rains became steady.

Data were collected on growth characteristics, size germination and tiller counts at specific growth stages, and stalk characteristic (number/stool, stalk length and diameter. Internodes number and length). At flowering, data were also collected on flowering behaviour (flowering versus non-flowering) and period of arrowing beginning from eight months after planting (MAP). Total soluble solids i.e estimate of sucrose in the juice) were collected on monthly basis until final harvest while at maturity with the aid of a hand refractometer. Other data collected included number of millable canes/stool and single stalk weight as well as cane yield/plot. cane yield/plot was later converted into tons/ha. The ratoon crop were thereafter maintained until the end of the growing season in 2012 and similar data as for the main crops were collected on the ratoon crops.

Pollen viability and fertility tests were conducted through confirmatory using the light microscope matured anthers on the spikelet's at shedding period were collected in sample bags and taken to the laboratory for examination. The anthers were treated with solution of absolute acetic acids and alcohol in ratio of 1:3 and placed inside Petri dishes with the solution for a period of one hour. The anthers were then removed from the solution and placed on slide and then squashed to release the pollen grains. After squashing few drops of stain lacto- phenol cotton blue were added to the squashed pollen grains for staining. The pollen grains with the stain were then covered with cover slips to prevent the pollen grains from displacement. The prepared slides were examined under light microscope for pollen grain fertility, the number of pollen grains in live microscopic fields were counted and sorted into fertile (fully round and dark in colour) and infertile pollen grains (clear empty and colorless). Pollen fertility was thereafter expressed as.

$$\text{Percentage (\%) fertility} \quad \frac{\text{No of fertile pollens}}{\text{Total of pollens}} \times 100$$

Based on the pollen fertility, the flowering genotypes were then classified either as males (≥40%) or female (≤40%).

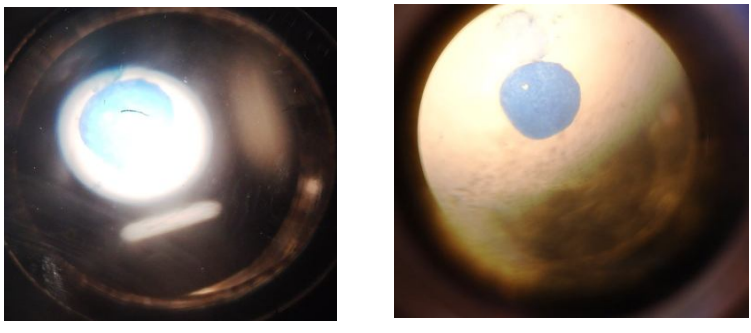

Plate 1: Infertile/defective pollen grain as observed in accession B78679 (Left) and fertile pollen grain in accession BJ82112 (Right) after stained with Lacto-phenol cotton blue

Data collected were subjected to analyses of variance and. Pertinent means were separated with the least significant difference (LSD) according to Steel and Terrie. (1980). Rank summation index (R∑I) was used to identify superior genotypes using the combination of cane yield and sucrose content. the procedure is represented

as RSI R(i) where R(i)j = observed ranking for the j traint for I clone 1.2… clones, I 1.2….m trait. Thus a clone ranked first in the two traits will have an index of two.

Results

The genotypes differed significantly for days to each of the flowering stages as shown in (Table 1) and formed three distinct groups with respect to days to pollen shedding. Eight (8) were early (EF), five (5) intermediate (MF) and seven (7) late flowering (IF) respectively, as shown in table (1) below:

Table 1: Days to Attaining Flowering Stages in Sixteen Exotic Sugarcane Germplasm Accessions and Four Check Varieties

Genotype	Initiation	Flagging	Emergence	Shedding	Classification +
KNB9211	309	316	323	330	Late
B80689	306	313	320	327	Late
B85877	288	295	302	309	Early
B74541	295	302	309	316	Mid
B93310	281	288	395	302	Early
D8415	289	296	303	310	Mid
DB8113	396	303	310	317	Late
KNB92101	296	303	310	317	Mid
B881607	280	287	294	301	Early
DB8687	291	298	305	312	Early
B78679	311	318	325	332	Late
B93638	309	316	323	330	Late
DB7869	302	310	317	324	Late
BJ82112	302	310	317	324	Late
B76621	286	293	300	304	Late
BT871646	296	303	310	317	Mid
ILS-001+	279	286	293	300	Early
ILS-002+	294	300	307	314	Mid
B47419+	299	286	293	300	Early
USRI/85/31+	286	293	302	307	Early
Mean	294	301	308	315	
F-Test	*	*	*	*	
LSD (∞0.05)	17.83	17.83	17.83	17.83	
% CV	3.67	3.59	3.56	3.45	

+; Checks

++: Early flowering (279-288 days): Mid-flowering (289-288 days) and Late-flowering (299-311 days) (unilorin 2011)

This information is very important in planning crosses either on the field or in the screen house. Examination of the pollen grains under the light microscope revealed defective and collapsed structures of pollen grains (i.e in viability) for some of the genotype. Notably among them were accessions KNB9211 and B78679 as well as variety ILS-002 (Plate 1, other accessions such as BJ 82112. B 881607 and ILS-001 produced viable pollen grains which absorbed the Lacio-phenol cotton blue stain and were fully round in shape.

Pollen production in the flowering genotypes ranged from very abundant (>600: Five microscopic fields) in accessions BJ 82112 KNB 9211 and B 80689 to as low as 63. Five microscopic fields in accessions B93638 and BT871646 as shown in (Table 2). Majority of the genotypes with low pollen production capacity had high percentage (%) of viable pollen grains which suggests an inverse relationship between pollen production capacity and fertility. However, this observation was not without exception because some accessions with high pollen production capacity (see for example. BJ82112 and B80689) had high pollen fertility while another accession with high pollen production (KNB9211) and few fertility as indicated by % viable pollen grains indicating. Male sterility. The genotypes with defective pollen grains can therefore be used as female in sugarcane hybridization programme as shown in table 2 below:

Table 2: Pollen Characteristics and Sexual Classification in Sixteen of Exotic Sugarcane Germplasm Accession and Four Check Varieties

Genotype	Total count	Fertile	Infertile	%fertility	Sexual classification
KNB9211	743	235	508	39.15	Female
B80689	600	501	99	58.30	Male
B85877	176	67	109	46.45	Female
B74541	85	46	39	52.37	Male
B93310	224	129	95	55.94	Male
D8415	290	122	168	51.35	Male
DB8113	468	331	137	70.47	Male
KNB92101	405	32	85	57.91	Male
B881607	213	51	70	55.76	Male
DB8687	249	150	95	65.17	Male

B78679	954	415	555	32.99	Female
B93638	63	49	13	86.44	Male
DB7869	86	24	62	62.29	Male
BJ82112	931	683	248	74.69	Male
B76621	454	268	246	60.27	Male
BT871646	63	40	24	59.36	Male
IL-S-001	209	134	86	52.56	Male
IL-S-002	598	271	327	36.71	Female
B47419	192	145	46	73.56	Male
USRI/85/31	531	412	119	72.49	Male
Means	372.1	217.1	156.5	58.20	
F-Test	ns	ns	ns	ns	
LSD (∞0.05)	122.29	148.88	130.95	46.46	
% CV					

+; ≤ = Female: ≥ = Male.

Sexual classification in sixteen (16) exotic sugarcane germplasm accessions and four (4) cheek varieties (unilorin 2011)

The trend in sucrose accumulation in the juice among the flowering (FI) and non flowering (NF) genotypes are presented in figure I. Sucrose content increased with sampling time regardless of the flowering behaviour except that there was a noticeable decline among the FI types especially at eleventh months after planting (MAP). However, the NI types as a group had higher sucrose content compared to the FL types throughout the period of sampling and also at harvest. The difference in sucrose content between NF and FI types ranged from 13.4% at the beginning of sampling to 13.6% at harvest but with the highest of 17.9% obtained at IIMAP.

Within the FI group, the trend in sucrose accumulation in the juice showed that the flowering (LF) types as a group was superior to the intermediate flowering (MF) types especially beginning from 9MAP (Figure 2). Surprisingly, the early flowering (EF) types had slightly higher sucrose content than the MF types beginning from 10MAP until harvest. However, differences between the groups were not significant probably because none of the genotypes had brix value lower than the acceptable minimum standard of ≥18 which is usually set as selection criterion for advancing sugarcane genotypes from the preliminary evaluation stage to advanced yield trails. For example, the difference between sucrose content at harvest between LF and EF was 2.0% and between LF and MF was 6.27% respectively while the difference between EF and MF was 4.3% as shown in figure 1 & 2 below:

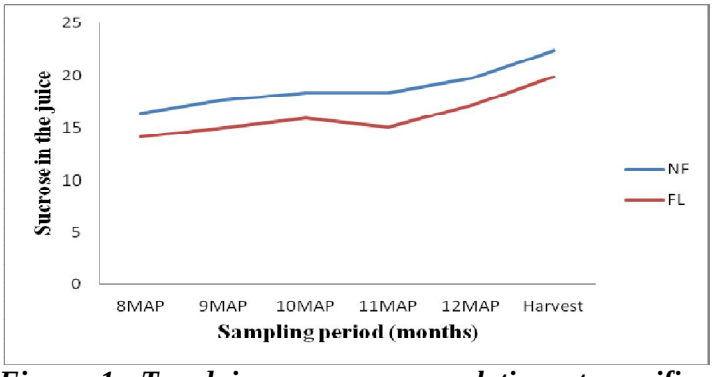

Figure 1: Trend in sucrose accumulation at specific sampling periods in non flowering (NF) and flowering (FL) sugarcane germplasm accessions.

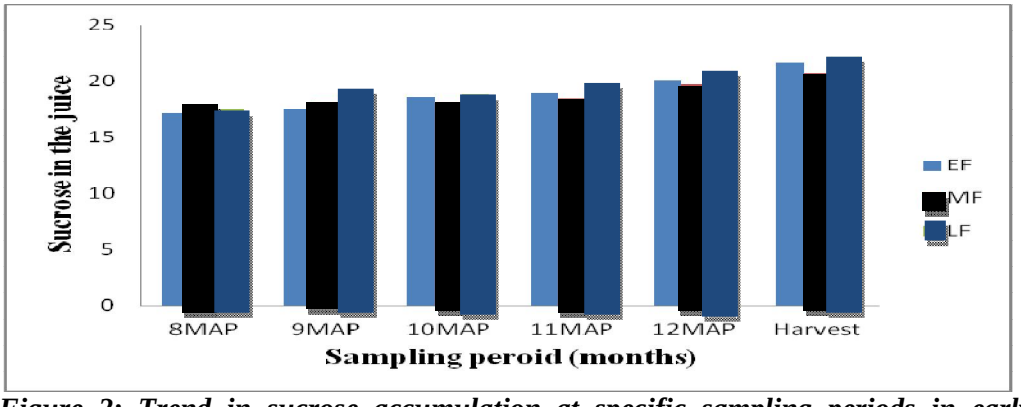

Figure 2: Trend in sucrose accumulation at specific sampling periods in early flowering (EF), mid-flowering (MF) and late flowering (LF) sugarcane germplasm accessions.

Means for cane yield and important yield components are presented in Table 3. The cropping cycles (CC) as well as the genotypes (G) differed significantly for all the traits. However GxCC interactive effects was significant only for brix at harvest. Differences among the genotypes for cane yield ranged from 22.11/ha in BR 8230 which was the lowest yielding genotype. The range in the means of millable canes was also large with a difference of 78 stalks between accessions KNB9252 (largest) and B62163 (lowest). However, there was no direct relationship between this trait and cane yield since accession B76251 with he highest cane yield had 47 millable canes which is just 50% of millable cane population in accession KNB9252.

Ranking of the genotypes on the basis of either cane yield or sucrose content in (table 4) revealed differential performance of the accessions with respect to each trait. Accessions B80689, BT74209 and BR8230 in that order were the top three yielding clones and were also superior to the best check (ILS-001) which ranked 9th overall for cane yield two accessions (B76251 and B80689) and a check variety (ILS-001) in that order were the best for sucrose content. however rank summation index (RSI) based on combination of cane yield and sucrose content revealed that five (5) accessions (B76251. B80689. KNB9211. D8415 and B85877) were superior to others as well as some of the check varieties used in this study two of the check varieties (ILS-001 and Co957) ranked third and sixth respectively based on R∑I for cane yield and brix as shown in table 3 below:

Table 3: Ranking of Thirty (30) Exotic Sugarcane Germplasm Accessions and Six (6) Check Varieties Based on Combination of Cane Yield and Sucrose Contents

Accessions/varieties	Cane yield/h	Sucrose content	∑RSI	Rank
D8415	99.792 (1)	22.8 (8)	9	1st
B80689	91.708 (11)	25.0 (1)	12	2nd
IL-S-001	92.500 (9)	23.5 (4)	13	3rd
DB85106	97.000 (4)	22.7 (10)	14	4th
B76251	91.708 (11)	23.2 (5)	16	5th
C0957	90.292 (14)	23.2 (6)	20	6th
B78697	98.000 (3)	21.7 (18)	21	7th
BT74209	68.417 (22)	23.8 (2)	24	8th
DB8113	99.750 (2)	20.8 (24)	26	9th
DB7869	96.375 (6)	21.5 (21)	27	10th
B93310	91.458 (13)	21.8 (15)	28	11th
B85877	94.292 (7)	21.3 (22)	29	12th
KNB9211	77.792 (19)	22.5 (11)	30	13th
USRI/85/31	96.750 (5)	20.5 (26)	31	14th
B991037	81.333 (18)	22.2 (14)	32	15th
B881607	85.292 (17)	21.8 (16)	33	16th
B77602	69.375 (21)	22.3 (13)	34	17th
B82233	49.875 (31)	23.0 (7)	38	18th
B82238	91.917 (10)	20.2 (28)	38	18th
BR8230	27.083 (36)	23.7 (3)	39	20th
BJ82112	50.583 (30)	22.8 (9)	39	20th
B8687	93.000 (8)	19.3 (33)	41	22nd
B47419	53.333 (29)	22.5 (12)	41	22nd
B79419	64.167 (23)	21.7 (19)	42	24th

B98653	89.2s50 (16)	19.8 (30)	46	25th
B74541	56.959 (27)	21.2 (23)	50	26th
KNB9252	90.125 (15)	18.5 (35)	50	26th
B63118	72.250 (20)	19.17 (32)	52	28th
B76621	29.167 (35)	21.8 (17)	52	28th
KNB92101	58.750 (26)	20.3 (27)	53	30th
BT871646	63.083 (24)	20.2 (29)	53	30th
B85266	38.083 (34)	21.5 (20)	54	32nd
C062175	41.250 (32)	20.7 (25)	57	33rd
B62163	59.792 (25)	17.8 (36)	61	34th
IL-S-002	54.667 (28)	18.7 (34)	62	35th
B93638	39.292 (33)	19.7 (31)	64	36th

Rank submission index of thirty (30) exotic sugarcane germplasm accessions and six (6) cheek varieties (unilorin 2011)

Discussion

Although flowering is an undesirable trait under commercial sugarcane production because it leads to progressive reduction in cane yield and sucrose contents if harvesting is delayed in such varieties (Nuss. 1977. Rao. 1977: Oworu. 1987: Fadayomi, R.O, Y.A Abayomi & G. Olaoye (1995). It is the primary tool employed by sugarcane breeders to evolve superior progenies intended as replacement of the existing cultivars. The accessions evaluated in this study particularly the flowering types, exhibited significant difference for almost all the characters measured or estimated (except pollen characterization) which is an indication of variability among them. This variability could be utilized in hybridization procedure aimed at the production of genetically diverged progenies from which selection of superior progenies for evaluation and future advancement as varieties could be practiced.

Pollen fertility in genotypes with high pollen production capacity was generally low. Suggesting an inverse relationship between the two characters. This observation agrees with an earlier report (Olaoye, 1996) which noted similar relationship between pollen production and pollen viability among earlier sets of sugarcane accessions evaluated under Ilorin conditions. Furthermore, Bull and Glaszeous (1979) noted that cultivars with low pollen viability can be used as female parents in hybridization with those having high viability as males in planned crosses. In other words, grouping the flowering accessions status in this study. This provides opportunity for utilizing them in hybridization programme. For example, accessions B80689. DB8687. DB7869, as well as varieties ILS-001 and USRI/85/31 were late female parents, while accessions KNB9211 and DB8687 were late maturing and male sterile in bi-parental crosses took advantage of their high yield potential at specific crosses. Alternatively, the male

parents could be used in polycrosses in conjunction with the female parents identified in this study and those already in the pool which were similar in maturity period, and not on extent of period of flowering.

Sucrose yield of a stem has been found to depend on biomass production and sucrose concentration which are interrelated processes Ebrahim, M.K, Z.O Zinghein, M.N El-Shourbagy, P.H More & E. Konor (1989) as well as the ability to accumulate high concentrations in harvested stem through the synthesis and breakdown of sucrose (Huber and Huber 1992). Individual genotypes used in this study, showed differences in their capacity to accumulate and synthesize sucrose as shown by the significant differences among them for this trait. The observed trend in sucrose accumulation between NF and FI as well as within the three sub-groups among the FI. Genotypes) EF. MF and LF) indicated that NF are likely to accumulate sucrose longer than FI and LF better than either MF or EF, the fact that many of the FF genotypes (see for example B 93310 and B 85877) showed superiority over other genotypes for sucrose content corroborate earlier findings of Olaoye (1999) that the ability to accumulate and synthesize sucrose may be genotype dependent.

As expected, there was an inverse relationship between sucrose content and cane yield which suggests that different ganes may be coding for the two characters in sugarcane. For example different genotypes exhibited superiority over others when selection of best candidates was based on either of the two characters. However, three (3) genotypes (B76251. B80689 and ILS-001) combined high cane yield with high sucrose content. incidentally, accessions B 80689 and ILS-001 were the flowering types and could be utilized in planned crosses. However, the variety ILS-001 which was bred under Ilorin conditions and released as a non flowering variety now flowered in the same locality, (Berding and Hurney, 2005: Ishaq and Olaoye 2010) observed that flowering in sugarcane i.e. (extent, intensity and sexuality) (maleness or femaleness) are usually variable especially at many tropical locations due to sub-optimal photoperiod and highly influenced by environmental conditions in a given locality.

Conclusion

The results obtained from this study revealed that many of the introductions were superior to the existing varieties for cane yield and sucrose content. this suggests the possibility of finding replacement to the existing low yielding varieties currently in cultivation on the estates. However, these superior genotypes will still need to undergo plantation testing in order to identify those that can be cultivated economically for a long time on the estate. Similarly, the flowering types which combined high cane yield with acceptable levels of sucrose in the juice could be used in hybridization to evolve progenies from which newer and superior varieties may be evolved in future.

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