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In vitro Germination of Pollen Grains and Growth of Pollen Tubes in Two Varieties of Sugarcane

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ABSTRACT

Ten growth media were tested in determining the *in vitro* germination and tube growth of two varieties of sugarcane (*Saccharum* sp.). It was found that the medium containing 30% sucrose modified with 0.03% magnesium and potassium respectively gave the best germination and tube growth. Thirty percent sucrose medium was found to be optimal for pollen grains germination of sugarcane varieties B49159 and B69691. B69691 consistently gave better pollen germination than B49159 in all the media used. Percentage initiated pollen grains increased with sucrose concentration while burst pollen grains decreased with sucrose concentration. Addition of magnesium and potassium appeared to inhibit burst pollen grains. Pollen tube growth was observed to be more sensitive to alterations to cluture medium than percentage of pollen germination. Germination was enhanced when various substances were added to the sucrose growth medium. The addition of 1% agar and 0.01-0.03% magenesium and potassium to the 30% basal sucrose medium was found to give good germination while tube growth was improved when 0.03% magnesium and potassium were added to the 30% basal sucrose medium. The implication of the findings for sugarcane improvement is discussed.

INTRODUCTION

Pollen viability in sugarcane (*Saccharum officinarum* L.) is of considerable theoretical and practical interest to sugarcane breeders. Discrepancy in environmental condition, especially low temperature conditions may decrease pollen viability so greatly that in cross pollinated plants fertilization may not occur. Under Nigerian and Beninoise conditions (e.g. rainfall, temperature and relative humidity) particularly in the southern part of these countries, flower initiation is promoted during the 4-5 months of growth which usually do not appear above the flag leaf until September-October (Oworu *et al.* 1988). Variation in the time of flowering between different sugarcane clones have been reported (Maun *et al.* 1969) and synchronization of flowering does not occur in several clones hence the crossing of one clone with another becomes problematic unless viable pollen grains are stored for this purpose.

Several methods have been postulated for determining the viability of pollen grains (Vasil, 1960; Pfahler, 1965). However, the results of testing these postulates do not always

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tally (Goss, 1968; Collins *et al.* 1973). There are three basic types of viability tests. The first is the use of vital strains such as the bromide or the tetrazolium chloride tests for dehydrogenase activity which are the most common methods on which pollen viability tests are based. However, Collins *et al.* (1973) postulated that much enzyme activity may be present beyond the viability of the pollen rendering results of such tests inconclusive. The second test is the direct method or the *in vitro* test in which the ability of the pollen grains to nick and cause seed set when placed on the stigmas is observed. However, the *in vitro* test becomes problematic when only incompatible stigmas are available, stray pollen cannot be controlled and a high level of selfing persists.

The *in vitro* germination of pollen grains is considered the easiest method because of the time requirement and the efficacy of the assay in detrmining its ability to fertilize and cause seed set. Results of study on this *in vitro* germination of pollen grains have been scanty and limited to a small number of *Graminae* such as *Setaria asphacelata* (De Bruyn, 1966 a, b); *Pennisetum typhoides* (Vasil, 1960); *Zea mays* (Cook and Walden, 1965, 1967).

The initial problems of the various viability tests have been the determination of the nutrient requirements for *in vitro* pollen grains germination and pollen tube elongation which vary from species to species. Most nutrient solutions have included a carbohydrate source-sucrose, boron and calcium ion (Brawbaker and Kwack, 1963; Cook and Walden, 1965). Information on sugarcane pollen grains germination are scanty and inconsistent (Weller, 1930; Sartoris, 1942).

This paper reports the results of experiments undertaken to develop an *in vitro* test for sugarcane pollen germination that could be utilized in determining pollen viability.

MATERIALS AND METHODS

The experiments were carried out in the Factory laboratory of the Save Sugar Company in the Republic of Benin during the 1987/88 cropping year. Pollens were collected from plants of B49159, a commercial sugarcane variety, and B69691, an F_1 hybrid. The plants from which pollen grains were collected were maked out in the field and the evening prior to pollen collection, panicle of each selected plant was vigorously shaken and brushed to remove old anthers and pollen. Since longevity of pollen is measured in minutes or hours, pollen was used immediately after anthesis which normally occurs during the early morning hours. The inflorescence of each seclected plant was enclosed in a paper bag and sealed with a rubber band, the previous evening prior to pollen collection. The next morning between 0700 and 0730 hours, the inflorescence was shaken before the removal of the paper bag, so that the falling pollen was collected in the bag. The cleaning of the collected pollen was accomplished by screening.

Pollen grains were gently dusted onto the test media and the number consistently kept low (about 150 grains/hanging drop) to minimize "population effects" (Brewbaker and Kwack, 1963). A hanging drop of the test medium was placed on a coverslip to which pollen was dusted and later inverted over a deep well slide. A drop of water was placed in the well to reduce evaporation from the drop of medium and the coverslip was sealed to the slide by mineral oil. Pollens were incubated for 3 hours at a relative humidity of 90%. Grmination was terminated by the addition of a formalin acetic acid (FAA) fixative. Tests were conducted at 25°C.

Three viewing fields of the microscope were made at 150x to obtain data on about 100 to 120 pollen grains. All pollen grains appearing in the microscopic field of vision were ascribed to one of the following classes :-

1. Burst-ungerminated pollen grains showing exudation of grain contents.

2. Initiated-pollen grains showing a protuberance less than one diameter of the pollen grain.

3. Germinated pollen grains showing growth of a pollen tube whose length exceeds the diameter of the pollen grains.

The pollen tube of germinated grains was measured using a graduated micrometer at 400 magnification. Distilled water and reagent -grade chemicals were used throughout the experiments. The media used were based on the modified synthetic media devised by Brewbaker and Kwack(1963).

Potassium and magnesium were provided in the form of KNO_3 and $MgSO_4$. $7H_20$ at 0.01%, 0.02%, and 0.03% respectively. The experiment was a factorial design arranged in random blocks with three replicates. All the treatment accounts were converted into percentage values of the total number of grains on the three fields of the slide. The percentage data were transformed to Arosin percentage for analysis.

RESULTS AND DISCUSSION

The Table 1 shows the percentages of germination, initiated and burst pollen grains in distilled water and increasing concentration of sucrose. In both varieties, germination was better in sucrose than in water. The optimum sucrose for the two varieties was 30% which improved the germinated pollens (48% for variety B 69691 and 37% for variety B 49159) and thereafter decreased.

Table 1. Effect of growth media on percentage of germinated, initiated and burst pollen grains in two varieties of sugarcane.

Variety	% Germinated pollen							% Initiated pollen							% Burst pollen						
	Distilled water	Sucrose concentration(%)						Distilled water	Sucrose concentration(%)						Distilled water	Sucrose concentration (%)					
		10	20	30	40	50	60		10	20	30	40	50	60		10	20	30	40	50	60
B 69691	12	23	36	48	33	30	25	20	25	29	33	35	42	47	44	38	30	27	25	21	20
B 49159	10	19	30	37	29	24	19	24	31	36	41	61	54	64	64	34	27	19	16	15	13
LSD (P=0.05)				CV							CV							CV			
Between varieties		0.56		1.20					0.63		0.91					0.48		1.00			
Between treatments		0.63		1.37					1.17		1.81					0.88		0.52			
Variety x Treatment interaction		1.46		3.18					1.65		2.53					0.92		1.93			

Table 2. Percentage of germinated, initiated and burst pollen grains from two varieties of sugarcane in 30% sucrose + 1% Noble agar.

Variety	% Germinated pollen	% Initiated pollen	% Burst pollen
B 69691	80	47	19
B 49159	70	52	15
LSD (p=0.05)		CV	
Between varieties	-	0.96 1.15	
Between treatments	-	1.19 1.42	
Varieties xTreatment interaction	-	1.67 2.00	

The pollen grains of B 69691 germinated better than those of B 49159 either in water or sucrose. Initiated pollen increased with sucrose concentration reaching its peak (47% for B 69691 and 64% for B 49159) at the highest (60%) concentration of sucrose. At less than 30% sucrose concentration, percentage of burst grains was significantly higher and percentage of initiated grains lower in the two varieties than at above 30% sucrose concentration. B 69691 gave the highest percentage burst grains (38%) at 10% sucrose concentration while the highest for B 49159 was 34% at 10% sucrose concentration. The results correspond with the findings of Maun *et al.* (1969) and Pfahler (1967) who reported that for germination and growth of pollen the concentrations of sucrose required 13% for bluegrass (*Pea pratensis* L.) and 15% for maize (*Zea mays* L.). It seems likely that sucrose, which has been found essential for *in vitro* germination of pollen from many species acts as a carbohydrate sucrose and an osmoticum to regulate diffusion.

The addition of 1% Noble agar appeared to inhibit burst pollen (Table 1). Significant difference ($P<0.05$) was observed between varieties and treatments and their interactions in the percentage germination, initiated and burst pollen grains when three concentrations of $MgSO_4$ were added to the 30% sucorse medium (Table 3). The highest percentage germination of 82% was recorded for B 69691 when pollen was immerged in 30% sucrose plus 0.03% $MgSO_4$ medium. The variety B 69691 consistently gave higher percentage germination than B 49159. The addition to potassium and magnesium at 0.03% concentration to the sucrose medium enhanced pollen germination when compared to the medium where agar was added. It may be postulated that the addition of agar to the basal sucrose medium may have altered the physical characteristics of the medium which presumably may control the degree of embedding of the pollen grains on the surface and which regulate the amount of oxygen and the solution absorbed by the pollen grain. This postulate was corroborated by work of Johir and Vasil (1961) who found that the addition of agar altered the physical and chemical composition of the medium. Germination and initiated pollen increase with the addition of magenesium and potassium concentrations (Tables 4,5). These findings are in agreement with other workers who observed that the addition of other elements to the external medium increased the percentage germination and pollen tube length. Pfahler (1965) reported that the adidtion of boric acid enhanced pollen germination of rye (*Secale cereale* L). Brewbaker and Kwack (1964) also reported that calcium considerably enhanced the *in vitro* germination of pollen tube growth in a number of speices.

Table 3. Percentages of germinated, initiated and burst pollen grains from two varieties of sugarcane in media containing 30% sucrose and three different concentrations of $MgSO_4$.

Variety	Medium	% Germinated pollen		% Initiated pollen		% Burst pollen	
B 69691	30% Sucrose	50		35		30	
	30% Sucrose + 0.01% $MgSO_4$	61		38		24	
	30% Sucrose + 0.02% $MgSO_4$	71		42		21	
	30% Socrose + 0.03% $MgSO_4$	82		49		16	
BB 49159	30% Sucrose	40		44		21	
	30% Sucrose + 0.01% $MgSO_4$	51		46		21	
	30% Sucrose + 0.02% $MgSO_4$	59		52		18	
	30% Socrose + 0.03% $MgSO_4$	67		62		13	
LSD (P=0.05)			CV		CV		CV
Between varieties		1.25	0.68	0.56	0.71	0.42	1.20
Between treatments		1.01	0.97	0.76	0.98	0.59	1.70
Variety x Treatment interaction		1.43	1.37	1.09	1.04	0.85	2.45

Table 4. Percentages of germinated, initiated and burst pollen grains from two varieties of sugarcane in 30% sucrose + three concentrations of KNO_3.

Variety	Medium	% Germinated pollen		% Initiated pollen		% Burst pollen	
B 69691	30% Sucrose	52		35		29	
	30% Sucrose + 0.01% KNO_3	48		36		21	
	30% Sucrose + 0.02% KNO_3	61		40		17	
	30% Socrose + 0.03% KNO_3	65		46		16	
B 49159	30% Sucrose	37		44		22	
	30% Sucrose + 0.01% KNO_3	43		45		16	
	30% Sucrose + 0.02% KNO_3	45		50		13	
	30% Socrose + 0.03% KNO_3	50		54		11	
LSD (P=0.05)			CV		CV		CV
Between varieties		0.75	0.86	0.45	0.60	0.50	1.61
Between treatments		1.06	1.22	0.65	0.86	0.73	2.33
Variety x Treatment interaction		1.50	1.72	0.92	1.23	1.03	3.27

Table 5. Effect of various media on % germination of sugarcane pollen grains from two sugarcane varieties.

Medium	% Germination		Mean
	Variety B 69691	Variety B 49159	
30% Sucrose + 0.03% $MgSO_4$ + 0.03% KNO_3	84	72	78 a*
30% Sucrose + 1% Noble agar	76	74	75 b
30% Sucrose + 0.03% $MgSO_4$	82	67	74 b
30% Sucrose + 0.02% $MgSO_4$	71	59	65 c
30% Sucrose + 0.03% KNO_3	65	50	57 d
30% Sucrose + 0.01% $MgSO_4$	61	51	56 d
30% Sucrose + 0.02% KNO_3	61	45	53 e
30% Sucrose + 0.01% KNO_3	54	38	46 f
30% Sucrose	46	39	43 g
Distilled water	12	10	11 h

* Means followed by the same letter(s) are not significantly different at the 0.05 level according to DNMR test.

Table 6. Percentage germinated, initiated and burst pollen grains from two varieties of sugarcane in 30% sucrose + 0.03% $MgSO_4$ + 0.03% KNO_3.

Variety	% Germinated pollen	% Initiated pollen		% Burst pollen
B 69691	81	50		16
B 49159	69	56		12
LSD (P=0.05)			CV	
Between varieties	-	0.27	0.30	-
Between treatments	-	0.84	0.98	-
Variety x Treatment interaction	-	1.19	1.39	-

Table 7. Pollen tube length (microns) of germinated pollen grains of two varieties (B 69691 & B 49159) and 30% sucrose + 0.03% $MgSO_4$ + 0.03% KNO_3 and 30% sucrose + 1% Noble agar.

Medium	Pollen tube length (microns)	
	B 69691	B 49159
30% sucrose + 0.03% $MgSO_4$ +0.03% KNO_3	245	215
30% sucrose + 1% Noble agar	202	178
LSD (P=0.05)		CV
Between varieties	5.86	3.09
Between treatments	5.86	3.09
Variety x Treatment interaction	8.29	4.37

Table 8. Effect of hydrogen ion (pH) concentration on % pollen germination in 30% sucrose + 0.03% $MgSO_4$ + 0.03% KNO_3.

Variety	Hydrogen ion concentration (pH)								
	4.0	4.5	5.0	5.5	6.5	7.5	8.0	8.5	9.0
B 69691	0	42	83	68	47	26	17	-	-
B 49159	0	37	77	62	43	22	13	-	-
LSD (P=0.05)								CV	
Between varieties					1.28			1.66	
Between treatments					2.22			2.89	
Variety x Treatment interaction					3.15			4.08	

However, the addition of magnesium and potassium to the 30% sucrose medium decreased burst pollen (Tables 1,6). The difference in the percentage germination between the two varieties may be attributed to the pollen source and genotypes. The variety B 69691 is a clone of *Saccharum spontaneum* while B 49159 is a clone of *Saccharum officinarum.* It may thus be postulated that pollen garins from *S. spontaneum* gave better germination in almost all the media tested than pollen from *S. officinarum* (Tables 1,6). Similar results were obtained by Pfahler (1970) who found differences between pollen genotype and sucorse in maize pollen germination. The addition of 0.03% magensium and potassium to the 30% sucrose medium enhanced tube growth. In the two media tested (30% sucrose+0.03% $MgSO_4$+0.03% KNO_3 and 30% surcorse +1% Noble agar) B 69691 had higher tube growth than B 49159 (Table 7).

The effect of pH on percentage germination when pollens from the two varieties were immersed in 30% sucrose+0.03% $MgSO_4$+0.03% KNO_3 medium (Table 8). There were significant differences in germination between varieties, treatments and their interactions. A high germination percenatge at pH 5.0 was followed by a decrease in germination as pH increased. Results show that pollens germinate and grow best in acidic medium. At pH 7.0-7.5 shorter tube length was observed and at pH 8.0 germination appeared abnormal with bulb shaped tube (Table 7). The optimum pH for pollen germination in the two varieties was 5.0. Pollen tube of B 69691 was consistently longer than that of B 49159 irrespective of the medium use (Table 7). The differences in tubes length between the media may be due to the addition of the agar to the sucrose medium. The addition of 0.03% magnesium and potassium to the sucrose medium allowed the pollen tube to elongate to about 245 microns, approximately five times the diameter of the average sugarcane pollen grain (50 microns). This result is corroborated by the findings of Maun *et al.* (1969) who reported that the addition of boric acid and calcium nitrate at 5 and 10 ppm respectively allowed the pollen tube of bluegrass pollen (*Pea pratensis* L.) to elongate to about 245 microns, 10 times the diameter of the bluegrass pollen grain. Wallace and Karbani (1968) also reported a pollen tube length of 200-250 microns for oat (*Avena byzantina* C. Koch) pollen when germinated on a medium consisting of 13g sucrose, 1.7g raffinose, 20mg boric acid, 25mg calcium nitrate, 3g bacto-agar and 85ml double distilled water.

Brewbaker and Kwack (1963) reported a "population effect" of pollen in small populations of pollen grew poorly under conditions that supported excellent growth of large populations. According to the authors, pollen requirement for calcium was suppplied by the calcium content of the pollen grains when large populations were germinated. In this experiment, population effect was not studied. It is suggested that *in vitro* method of pollen germination discussed here should be a veritable tool for the sugarcane breeder to determine the viability of pollen grains necessary in hybridization particularly in the bi-parental cross in a sugarcane breeding programme.

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