African Journal of Tropical Agriculture Vol. 1 (1), pp. 001-006, September, 2013. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Banana nectar as a medium for testing pollen viability and germination in *Musa*

Moses Nyine[1] and Michael Pillay*[2]

[1]International Institute of Tropical Agriculture (IITA), P.O Box 7878, Kampala, Uganda.
[2]Vaal University of Technology Private Bag X 021 Vanderbijlpark 1900, South Africa

Accepted 4 September, 2013

A quick and reliable method for evaluating pollen quality is essential in a breeding program, especially in a crop such as banana that is characterized by high male and female sterility. In this study the germination and viability of banana pollen was evaluated in a sucrose solution and diluted banana nectar. Twenty banana accessions were used to evaluate pollen germination in the two media after 3 and 24 h. Nineteen genotypes (95%) showed significantly higher pollen germination potential (PGP) in diluted nectar than in 3% sucrose solution. The accession TMB2x 8075 - 7 showed no significant pollen germination in nectar and sucrose. Eleven genotypes (55%) showed significant increase in pollen germination by increasing the time of incubation whereas pollen germination for nine genotypes (45%) was not affected by increase in incubation time. Nectar from different banana clones influenced pollen germination suggesting a genotype effect for pollen germination in *Musa*.

Key words: Banana, pollen germination, sucrose solution, diluted nectar.

INTRODUCTION

Banana is an important staple food crop and a source of income for millions of people in the tropical and sub-tropical regions of the world. Despite its importance, banana production has declined over the past 20 to 25 years due to pests and diseases. Since many of the rural poor depend on bananas for food and cash income, a decline in banana production leads to food insecurity and threatens the livelihoods of vulnerable groups. Genetic improvement of bananas and plantains is a challenging task because of their extremely low seed set rate in conventional crossbreeding schemes (Pillay et al., 2002). Most cultivated bananas are triploid ($2n = 3x = 33$) and are highly female and male sterile. Seed set in conventional crosses varies both among different banana cultivars and during different periods of the year (Ssebuliba et al., 2006). The initial steps in conventional cross-breeding programs in banana are hybridization and selection of recombinants at the diploid level (Novak, 1992). Improved, disease resistant and pollen fertile diploids are crossed with triploid (3x) varieties to produce tetraploid (4x) selections. The selected tetraploids are then crossed with improved diploids to produce secondary triploids (Pillay et al., 2002). The above crossing scheme also produces diploids that could be selected for use as male parents if they contain traits of interest for the breeder. Therefore, it is essential to ascertain the pollen fertility of these diploids. In addition, in an attempt to explain the variation in seed set rates observed during conventional cross-breeding schemes, breeders have taken keen interest in assessing the viability of pollen used in pollination (Stone et al., 1995).

A quick and reliable method of testing pollen viability is essential to study environmental factors that affect pollen development, to identify male sterility and to determine the optimum time for pollination (Adhikari and Campbell, 1998). *In vitro* pollen development has drawn considerable interest since novel applications such as genetic transformation of microspores have been successfully used to generate transgenic plants in *Nicotiana glutinosa* (van der Leede-Plegt et al., 1995), *N. tobacum* (Touraev et al., 1995, 1997; Aziz and Machray, 2003) and *Pinus sylvestris* (Aronen et al., 2003). A reliable method of pollen viability and germination also paves the way for *in vitro* pollination of isolated ovules, especially in banana that is fraught with pre- and post-zygotic barriers of inco-

*Corresponding author. E-mail: m.pillay@yahoo.co.uk.

Former address: International Institute of Tropical Agriculture. P. O. 7878, Kampala, Uganda

Table 1. Genotypes used in pollen germination studies in banana.

S/N	Genotype	Genome
1	*M. balbisiana*	BB
2	*M. acuminata*	AA
3	TMB2x2658S-17	AA
4	TMB2x 2975S-17	AA
5	TMB2x 2920S-7	AA
6	TMB2x 8075-7	AA
7	TMB2x 2920S-8	AA
8	TMB2x 2926S-5	AA
9	TMB2x 2975S-18	AA
10	TMB2x 2975S-46	AA
11	'Sukali ndizi'	AAB
12	TMB2x 2658S-57	AA
13	TMB2x 2868S-7	AA
14	TMB2x 2920S-10	AA
15	TMB2x 2920S-9	AA
16	TMB2x 2975S-25	AA
17	TMB2x 2975S-38	AA
18	TMB2x 2658S-19	AA
19	TMB2x 8532-1	AA
20	TMB2x 5265-2	AA

mpatibility.

Pollen viability can be determined by staining with nuclear and vital dyes, by *in vivo* and *in vitro* germination tests or by analyzing the final seed set (Hesslop-Harrison et al., 1984). Several dyes such as 1% 2, 5 diphenyl tetrazolium bromide in 5% sucrose (MTT) (Khatum and Flowers, 1995; Norton, 1966), 2, 3, 5 triphenyl tetrazolium chloride (TTC) (Oberle, 1953), X-Gal aniline blue in lactophenol, acetocarmine (Asghari, 2000) and Alexander's procedure (Alexander, 1969) have been used to differentiate viable and non-viable pollen. One of the biggest disadvantages with most staining techniques in assessing pollen viability is that most dyes stain both viable and non-viable pollen (Kapyla, 1991; Parfitt and Ganeshan, 1989; Khatum and Flowers, 1995; Sedgly and Hardarb, 1993). Only two techniques; (i) the new peroxidase test, and (ii) MTT (Rodriguez-Riano and Dafni, 2000) are capable of differentiating viable from non-viable pollen.

Pollen viability rates observed with the different staining techniques do not correlate well with *in vitro* germination rates of fresh pollen (Rodriguez-Riano and Dafni, 2000) suggesting that not all viable pollen is capable of germinating. Therefore, determination of pollen germination potential is a good way of selecting improved, disease resistant and pollen fertile diploids based on the assumption that pollen capable of germination would be fertile (Barrow, 1983). The most popular medium for *in-vitro* germination of banana pollen has been a sucrose solu tion (Brewbaker and Kwack, 1963). The objectives of this study were (i) to compare the rate of banana pollen germination in a sucrose solution versus a 1:9 diluted banana nectar with two incubation periods of 3 and 24 h, and (ii) to compare the effect of different sources of banana nectar on pollen germination.

MATERIALS AND METHODS

Pollen germination was assessed in twenty banana geno-types (Table 1) obtained from the IITA research fields at Namulonge, in Uganda. Pollen was germinated in two media: (i) a 3% sucrose solution containing 0.01 g H_3BO_3 , 0.25 g $MgSO_4.7H_2O$, 0.25 g KNO_3 and 0.4 g $Ca(NO_3)_2$, and (ii) a 1:9 v/v banana nectar diluted with de-ionized water.

Nectar was obtained from the male flowers of TMB2x8075-7, TMB2x2920S- 10 and TMB2x2975S-25. The 1:9 dilutions had a Brix of 2.8% when measured with a refractometer (ATC-1, ATAGO Co. Ltd, Japan) and a pH 6.5 at room temperature. Male flowers were collected from newly opened bracts between 8:30 am and 10:00 am. Pollen was dusted onto a cover glass so that a uniform spread was obtained. Three to four drops of the germination medium were placed on a clean glass slide with a Pasteur pipette and the cover glass was carefully inverted on the medium without trapping air. This method ensured that the pollen hangs over the germination medium. The slides were viewed under a light microscope under 40 x magnification. Fields with well-distributed pollen with high pollen counts were selected and marked with circles. For each genotype, two slides were prepared per germination medium and six fields were selected per slide. The slides were placed horizontally on a slide rack that was placed in a moist glass humidity chamber and incubated at room temperature for 24 h. The number of germinated and non-germinated pollen was recorded from the marked areas of each slide after 3 h and then after 24 h. The percent of germination was determined by the formula: Percentage germination = total germinated pollen/total pollen count x100.

To compare the effect of different sources of nectar on pollen germination, banana nectar was obtained from the two ancestral species, *Musa acuminata* spp. *burmannicoides*, 'Calcutta 4'(AA) and *M. balbisiana* (BB), an East African Highland variety, 'Tereza' (AAA) and four banana hybrid clones, TMB2x2868S-7, TMB2x2975S-18, TMB2x8075-7 and TMB2x2975S-25. For purposes of comparison pollen germination counts were done only after 3 h of incubation. The data obtained was analyzed using SAS GLM Procedure, version 8 (SAS, 1999).

RESULTS AND DISCUSSION

Pollen germination in different media and at different incubation periods

With the exception of TMB2x8075-7, the rate of pollen germination was significantly higher in diluted nectar than in the 3% sucrose solution in all the banana genotypes (Table 2). The rate of pollen germination in the diluted nectar ranged from 60 to 97% for most of the genotypes except in TMB2x2920S-8 in which germination was 44.2%. Pollen germination in the sucrose solution ranged from 17 to 50%.

One of the insights obtained from this study is that diluted banana nectar appears to be a better medium for determining pollen germination and viability in *Musa* than

Table 2. Mean pollen germination rates (%) of banana genotypes observed in sucrose (S) and diluted nectar (N) after 3 and 24 h incubation.

Genotype	Parents	Effect of medium			Effect of incubation period		
		Medium	Germ LSmeans	Pr > 0.05	Hours	Germ LSmeans	Pr > 0.05
TMB2x 2658S-17	8075-7 x	N	94.18	<.0001	3	68.18	0.2442
	6142-1	S	46.34		24	72.33	
TMB2x 2658S-19	8075-7 x	N	84.61	<.0001	3	50.77	<.0001
	6142-1	S	43.71		24	77.54	
TMB2x 2658S-57	8075-7 x	N	96.96	<.0001	3	83.54	0.0014
	6142-1	S	79.34		24	92.76	
TMB2x 2868S-7	8075-7 x	N	78.67	0.0017	3	51.61	<.0001
	6142-1	S	55.72		24	82.77	
TMB2x 2920S-10	8075-7 x	N	94.58	<.0001	3	77.73	0.0261
	6142-1	S	69.38		24	86.23	
TMB2x 2920S-7	8075-7 x	N	92.56	<.0001	3	60.14	0.2981
	6142-1	S	33.44		24	65.86	
TMB2x 2920S-8	8075-7 x	N	44.21	0.0042	3	35.73	0.4087
	6142-1	S	30.95		24	39.42	
TMB2x 2920S-9	8075-7 x	N	97.06	<.0001	3	79.36	0.2274
	6142-1	S	67.04		24	84.74	
TMB2x 2926S-5	8075-7 x	N	95.02	<.0001	3	56.10	0.6230
	6142-1	S	18.66		24	57.58	
TMB2x 2975S-17	8075-7 x	N	93.47	<.0001	3	61.88	0.0014
	6142-1	S	41.68		24	73.28	
TMB2x 2975S-18	8075-7 x	N	97.28	<.0001	3	63.87	0.7582
	6142-1	S	32.07		24	65.47	
TMB2x 2975S-25	8075-7 x	N	92.90	<.0001	3	71.44	0.1659
	6142-1	S	56.49		24	77.94	
TMB2x 2975S-38	8075-7 x	N	95.73	<.0001	3	64.19	0.1507
	6142-1	S	39.13		24	70.67	
TMB2x 2975S-46	8075-7 x	N	92.17	0.0024	3	80.84	0.0374
	6142-1	S	78.37		24	89.70	
TMB2x 5265-1		N	89.71	<.0001	3	57.89	0.0016
		S	35.21		24	67.03	
TMB2x8075-7	SH3362 x C4	N	72.25	0.1315	3	66.29	0.1970
		S	65.79		24	71.75	
TMB2x 8532-1		N	81.96	<.0001	3	41.39	<.0001
		S	17.00		24	57.57	
M. acuminata	Wild banana	N	83.15	<.0001	3	55.34	<.0001
		S	54.90		24	82.71	
M. balbisiana	Wild banana	N	60.90	<.0001	3	35.19	0.0050
		S	18.50		24	44.22	
Sukali Ndizi	Exotic	N	79.37	<.0001	3	61.09	0.0083
		S	52.02		24	70.29	

3% sucrose solution. The major components of nectar in most plants are sucrose, glucose, fructose, xylose (Nicolson and Van Wyk, 1998), various proteins (Peumans et al., 1997; Galetto and Bernardello, 2004) and organic acids (Sara et al., 2002). The nectaries in banana are located at the base of filaments of the male flowers.

The nectar is viscous and sweet due to presence of sugars. However, the exact composition of banana nectar is unknown. Due to its viscosity it can only be used as a pollen germination medium after dilution with sterile water. The diluted nectar can be stored at –20°C and used over a long time period. The Brix of the diluted ban-

Table 3. Analysis of variance showing effect of genotype, medium and incubation on pollen germination rate in *Musa*.

Source of variation	Df	MS	F-value	Pr > F
Genotype	19	8567.58	28.00	<.0001
Medium	1	364262.67	11900.28	<.0001
Time	1	32361.17	105.74	<.0001
r^2				0.65

df = degrees of freedom, r^2 = R-Square

ana nectar ranges from 2.2 - 3.2 and is similar to that of a 3% sucrose solution. However, it appears that bana-na nectar may contain other chemical components that stimulate pollen germination. Kwan et al. (1969) indicated that apart from boric acid that stimulates pollen germination in *Allium cepa*, gibberellic, succinic and fumaric acids also enhanced germination. It is possible that similar acids are also present in banana nectar thereby enhancing pollen germination. Further study is required to prove this assumption.

Effect of incubation time on pollen germination

This study showed that in 55% of the genotypes pollen germination rates increased significantly as the incubation time was increased (Table 2). The biggest increase of 31% was observed in TMB2x2868S-7 after 24 h and the lowest increase of 1.6% was observed in TMB2x2975S-18. Pollen germination rate after 24 h for the wild species *M. acuminata* (Calcutta 4) was 82.7% and *M. balbisiana* was 44.2%. Pollen viability rates for *M. acuminata* and *M. balbisiana* in Australia was given as 71% and 98%, respectively (Fortescue and Turner, 2004). While the pollen germination rate of *M. acuminata* in this study is similar to that of the Australian study, the rate of pollen germination for *M. balbisiana* differed significantly. It is known that pollen viability is greatly influenced by temperature, humidity, genotypic differences, vigor and physiological stage of the plant and the age of the flower (Shivanna and Johri, 1989). This could account for the differences observed in the pollen germination rate of *M.balbisiana* in the two studies. The 70.3 % germination rate for 'Sukali ndizi' (AAB) was similar to that of 'Calcutta 4' and supports the findings of Fortescue and Turner (2004) that genomes (A or B) do not affect pollen viability in *Musa*.

The increased pollen germination rates observed after 24 h of incubation suggests that a certain degree of dormancy may be present in viable pollen. A prolonged incubation period is necessary to break down the dormancy. Alternatively, some of the pollen may not have reached physiological maturity and a longer incubation period is required before they germinate. Pollen germination in some plants is a controlled process. Pollen germination studies in *Arabidopsis* showed that pollen not expressing any apyrase were unable to germinate. This revealed a novel role for apyrases in plants and identified an unexpected enzyme constituent required for sexual reproduction (Steinebrunner et al., 2003).

This study also showed that the pollen germination rate in TMB2x 8075-7 was not affected by media or incubation time. TMB2x8075- 7 is a progeny of a cross between SH3362 (from the breeding program of the Fundacion Hondurena de Investigacion Agricola, Honduras) and 'Calcutta 4'. It was interesting to note that eight genotypes that resulted from crosses in which TMB2x8075-7 was the male parent also showed no increase in germination rates as the incubation period was increased. This may suggest that pollen germination potential in *Musa* is, perhaps, genetically controlled and inherited from the male parent. It is known that other characteristics such as pollen color (Mehlenbacher and Smith, 2002) and pollen size (Ortiz, 1997) are also genetically controlled in plants.

Germination potential as been defined as the ability of viable pollen to germinate under suitable germination conditions (Fortescue and Turner, 2004). This study showed that genotype, medium and incubation time had a highly significant effect ($P<0.05$) on pollen germination potential. The mean germination rate of pollen in banana nectar (84.2%) was almost twice the rate obtained with 3% sucrose (47.4%). However, our results also revealed that the three variable factors (genotype, medium, incubation time) accounted for only 65% of the variability on pollen germination potential (Table 3). The remaining 35% may be accounted for by other factors such as humidity, temperature (Wang et al., 2004), photo conditions and pollen harvesting time (Shepherd, 1960) that also affect pollen germination potential (PGP).

The mean pollen germination rates in diluted nectar obtained from different banana clones and how they influenced pollen germination in different clones is compared in Table 4. Out of twenty-one possible comparisons, only eight (38%) showed that PGP depended on nectar source while the remaining thirteen (62%) had similar PGP irrespective of the nectar sources. This difference is perhaps due to differences in microclimatic conditions between the slides. In conclusion, this study showed that diluted banana nectar regardless of its source is a better medium to determine pollen germina-tion and viability rates in *Musa* than a 3% sucrose solution.

Table 4. Comparison of mean (%) germination in seven nectar sources.

Nectar Source	Mean pollen germination rates	TMB2x 2868S-7	TMB2x 2975S-18	TMB2x 2975S-25	TMB2x 8075-7	*M. acuminata*	*M. balbisiana*	'Tereza'
TMB2x 2868S-7	59.40	-	**0.0074**	0.4851	0.1433	0.2824	**0.0285**	**0.0031**
TMB2x 2975S-18	69.05		-	0.0629	0.2192	**0.0002**	0.6203	0.7577
TMB2x 2975S-25	62.03			-	0.4864	0.0861	0.1638	**0.0324**
TMB2x 8075-7	64.65				-	**0.0115**	0.4626	0.1269
M. acuminata	55.54					-	**0.0012**	**<.0001**
M. balbisiana	67.28						-	0.4237
'Tereza'	70.16							-

Bold value indicate significant difference in percentage germination

ACKNOWLEDGEMENT

This research was supported, in part, with funding from the Directorate General for International Co-operation (DGIC), Belgium.

REFERENCES

Alexander MP (1969). Differential staining of the aborted and non aborted pollen. Stain Technol. 44: 117-122.

Aronen TS, Nikkanen TO, Haggman HM (2003). The production of transgenic Scots pine (*Pinus sylvestris* L.) via the application of transformed pollen in controlled crossings. Transgenic Res. 12: 375–378.

Asghari J (2000). Estimation of pollen viability of Metsulfuron treated dyers woad (*Isatis tinctoria*) for herbicide efficacy evaluation. J. Agric. Sci. Tech. 2: 85-93.

Aziz N, Machray GC (2003). Efficient male germ line transformation for transgenic tobacco production without selection. Plant Mol. Biol. 51: 203–211.

Barrow JR (1983). Comparisons among pollen viability measurements in cotton. Crop Sci. 23: 734-736.

Brewbaker JL, Kwack BH (1963). The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot. 50: 747-858.

Fortescue JA, Turner DW (2004). Pollen fertility in *Musa*: Viability in cultivars grown in Southern Australia. Aust. J. Ag. Res. 55: 1085-1091.

Galetto L, Bernardello G (2004). Floral nectaries, nectar production dynamics and chemical composition in Six *Ipomoea* species (Convolvulaceae) in relation to pollinators. Ann. Bot. 94: 269-280.

Hesslop-Harrison J, Hesslop-Harrison Y, Shivanna KR (1984). The evaluation of pollen quality and a further appraisal of the fluorochromatic (FAR) test procedure. Theor. Appl. Genet. 67:367-375.

Kapyla M (1991). Testing the age and viability of airborne pollen. Grana 29: 430-433.

Khatum S, Flowers TJ (1995). The estimation of pollen viability in rice. J. Exp. Bot. 46: 151-154.

Kwan SC, Hamson AR, Campbell WF (1969). The effects of different chemicals on pollen germination and tube growth in *Allium cepa* L. HortScience 94: 561-562.

Mehlenbacher SA, Smith DC (2002). Inheritance of pollen color in hazelnut. Euphytica 127: 303-307.

Nicolson SW, Van Wyk BE (1998). Nectar sugars in Proteaceae: Patterns and processes. Aust. J. Bot. 46: 489-504.

Norton RB (1966). Testing of plum pollen viability with tetrazolium salts. HortScience. 89: 132-134.

Novak FJ (1992). *Musa* (Bananas and Plantains). In: Hammerschlag FA, Litz RE (eds). Biotechnology of perennial fruit crops. CAB International University Press Cambridge. pp. 449-488.

Oberle GD, Watson R (1953). The use of 2,3,5 triphenyl tetrazolium chloride (ttc) in viability test of fruit pollen. Am. Soc. Hort. Sci. 61: 299-303.

Ortiz R (1997) Occurrence and inheritance of 2*n* pollen in *Musa.* Ann. Bot. 9: 449-453.

Parfitt DE, Ganeshan S (1989). Comparison of procedures for estimating viability of *Prunus* pollen. HortScience 24: 354-356.

Peumans WJ, Smeets K, Van Nerum K, Van Leuven F, Van Damme EJM (1997). Lectin and alliinasse are the predominant proteins in nectar from leek (*Allium porrum* L) flowers. Planta 201: 298-302.

Pillay M, Tenkouano A, Hartman J (2002). Future challenges in *Musa* breeding. In:Kang M (ed) Crop Improvement- Challenges in the twenty-first century. Food Products Press. New York. pp. 223-252.

Rodriguez-Riano T, Dafni A (2000). A new procedure to assess pollen viability. Sex. Plant Reprod. 12: 241-244.

SAS (1999) SAS Institute Inc. Cary, USA.

Sara CC, José OF, Isabel MPLVOF (2002). HPLC/UV determination of organic acids in fruit juices and nectars. Eur. Food Res. Technol. 214: 67-71.

Sedgly M, Hardarb J (1993). Pollen storage and breeding system in relation to controlled pollination of four species of *Acacia* (Leguminosae. Mimosiodeae). Aust. J. Bot. 41: 601-609.

Shepherd K (1960). Seed fertility of edible bananas. J. Hort. Sci. 35: 6-20.

Shivanna KR, Johri BM (1989). The Angiosperm pollen, structure and function. John Wiley and Sons.

Ssebuliba R, Talengera D, Makumbi D, Namanya P, Tenkuoano A, Tushemereirwe W, Pillay M (2006). Reproductive efficiency and breeding potential of East African highland (*Musa* AAA-EA) bananas. Field Crops Res. 95: 250-255.

Steinebrunner I, Wu J, Sun Y, Ashley C, Roux SJ (2003). Disruption of apyrases inhibits pollen germination in *Arabidopsis*. Plant Physiol. 131: 1638-1647.

Stone JL, Thomson JD, Dent-Acosta SJ (1995). Assessment of pollen viability in hand pollination experiments: a review. Am. J. Bot 82: 1186-1197.

Touraev A, Fink CS, Stoger E, Heberle-Bors E (1995). Pollen selection: a transgenic reconstruction approach. Proc. Natl. Acad. Sci. USA 92: 12165–12169.

Touraev A, Stoger E, Voronin V, Heberle-Bors E (1997). Plant male germ line transformation. Plant J. 12: 949–956.

van der Leede-Plegt LM, van den Ven BCE, Schilder M, Franken J, van Tunen AJ (1995). Development of a pollen-mediated transformation method for *Nicotiana glutinosa*. Transgenic Res. 4: 77–86.

Wang ZY, Ge Y, Scott M, Spangenberg G (2004). Viability and longevity of pollen from transgenic and nontransgenic tall fescue (*Fescue arundinacea*) Poaceae plants. Am. J. Bot. 91: 523-530.