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Male fertility in *Musa*: Pollen quality in diploid banana hybrids

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Abstract

Banana production in the East African region is constrained by a variety of pests and diseases. Host plant resistance is the most convenient and effective intervention to circumvent these constraints in banana production. However, male fertility is a major limitation in the genetic improvement of bananas. Pollen quantity and quality are the key attributes in selecting male parents for successful banana crosses. Pollen was collected from 22 candidate diploid banana hybrids and 6 diploid genotypes previously, successfully used in breeding programs. It was examined for pollen quantity, staining with Iodine Potassium Iodide (I_2KI, Lugol stain), and germination frequency on a nectar-water medium. The traits under examination varied significantly ($P \leq 0.05$) among diploid banana hybrids. I_2KI-stained pollen frequency ranged between 12.96% for hybrid '12506S-1' to 100% for '3162K-1', while pollen tube germination frequency ranged from 1.67% on '2858K-5' to 95.66% on *'Calcutta 4'*. Relative pollen quantity, I_2KI-stained pollen frequency, and pollen germination frequency were significantly, positively ($P \leq 0.05$) correlated. Five diploid banana hybrids, i.e. '12506S-29', 'Opp2-861', '3162K-3', '3146K-3', and '3146K-2', were selected as male parents to be incorporated into the breeding programs.

Key words: Banana breeding, I_2KI pollen staining, pollen germination

Introduction

Bananas and plantains, (*Musa sp*) are among the four most important global food commodities together with rice (*Oryza sativa* L.*)*, wheat (*Triticum aestivum* L.*)*, and maize (*Zea mays* L.) (FAOSTAT, 2009). Like many crops of global importance, banana production is constrained by pests, diseases, and drought. A countrywide rapid rural appraisal (Gold *et al.*, 1993) revealed that banana weevil (*Cosmopolites sordidus*, Germ) and a complex of parasitic nematodes (*Meloidogyne spp*, *Radopholussimilis,* Thorne) are the major pests, while fungal leaf spots (*Mycosphaerella fijiensis*, Morelet.), *Fusarium* wilt (*Fusarium oxysporum*. f.sp. *cubense (E.F. Smith) Snyder & Hansen*), banana streak virus (*Badnavirus*) and *Xanthomonas* wilt (*Xanthomonas campestris* pv. *musacearum*) are the most important diseases. Banana producers have to deploy various cultural control practices

like weevil trapping, detrashing, de-leafing, debudding, and application of insecticides to overcome these production problems. However, host plant resistance to pests, diseases, and drought is the most feasible sustainable intervention. Wild and cultivated diploid bananas are widely used as male parents in banana breeding to generate genotypes with host plant resistance. Assessment of pollen fertility is indispensable for genetic crop breeding (Fortescue and Turner, 2004). Therefore, studies of pollen fertility in *Musa* are essential for selecting male parents in banana breeding.

Pollen quantity and quality are key attributes of male parents for successful banana crosses (Marrewijk, 1994; Dumpe and Ortiz, 1996). Genetic and environmental factors have been reported to affect the quality and quantity of pollen grains in banana genotypes (Krishnakumar *et al.*, 1992). To study male fertility in bananas, both quantity and quality must be determined reliably by either staining pollen with dyes or *in vitro* germination assays. Staining techniques are used to determine physiological and/ or structural integrity of pollen grains. The mode of action of each stain depends on molecules of the pollen grain to which it binds. Molecules of the pollen grain to which commonly used stains bind include the cytoplasm, nucleic acids, cellulose and starch granules (Alexander, 1969; Ashagari, 2000; Scott, 2001). Iodine Potassium Iodide (I_2KI**)** interacts with the coil structure of starch and distinguishes the quality of pollen grains based on the presence or absence of starch granules. This stain is most suitable for banana pollen quality studies since well formed banana pollen grains are filled with starch which provides nutrition for pollen tube growth. The latter is destined to grow across long tubular flowers with long styles. A lot of energy is required for the pollen tube to fulfill successful fertilization (Shepherd, 1960). The pollen germination is a convincing indication of the actual fertility of the pollen grains (Sharma and Sharma, 1972).The aim of this work was to identify potential male parents to be used in a banana breeding program.

Materials and methods

Using a Partec 1 ploidy analyser, 28 diploid banana genotypes were selected for studies on male fertility from the breeding fields and the banana germplasm collection at the National Agricultural Research Labaratories, (NARL), Kawanda in Uganda, during the second rainy season (September) of 2010. These genotypes included one hybrid obtained from the open pollination of diploid banana 'OPP1861', 3 hybrids from the inter-diploid crosses of '8075-7', '7197-2' and 'SH3362' and 17 diploid hybrids obtained from tetraploid by diploid crosses of, 'FHIA17', '917K-2', '401K-1', 222K-1' '1438K-1' and '365K-1'. Six diploids, i.e. '8075-7', '7197-2', 'OPP1861', '5105-1', '861S-1' and 'Calcutta 4' which have been used successfully as male parents by the breeding programs at IITA and NARO in Uganda were also included. The study was conducted at the National Agricultural Research Laboratories, (NARL), Kawanda, $0^0$25'N $32^0$32'E, 1190 m above sea level, with a moist sub-humid climate and a mean annual rainfall of 1250 mm per annum, bi-modally distributed; the main wet season being March-May and uncertain short rains in September-November.

Pollen quantity

Eight to ten (8-10) anthers were excised from the male bud of each genotype (early in the morning 7-8 a.m.) and rated for pollen quantity production using a modification of the method developed by Mukasa and Rubaihayo (1993) on a scale of 0-4, i.e. 0 - no pollen, 1 - low pollen, 2 - little pollen, 3 - moderate pollen, and 4 - much pollen. The data was converted to a range with a maximum of 100 using the formula: y' = ((y-a)/b)*100, where y' is relative pollen quantity, y the observed pollen rate, a the lowest score on the scale, and b the range of the scale.

Pollen staining with IKI_2

Pollen from 8 to 10 anthers collected between 7 and 8 a.m. was immediately scraped onto microscope slides, separately for each anther. The Iodine Potassium Iodide (I_2KI) stain (containing 0.5 g of iodine (I_2) and 1 g of potassium iodide (KI) dissolved in 100 ml of de-ionized distilled water) was used to stain starch in pollen grains on a microscope slide. The percent pollen filled with starch was estimated by counting the stained pollen after 2-5 min under a light microscope at x100 magnification. About 20 pollen grains from 6 selected areas were assessed from each of the 8-10 slides for each genotype. Only pollen grains filled with starch stained immediately black with I_2KI solution (Scott, 2001); the percentage of stained pollen grains was calculated from the pollen counts.

Pollen tube germination

For each genotype, pollen obtained from the excised anthers was dusted on cover slip and placed on a clear microscope slide where a drop of nectar in water (1:9 v/v) had been placed. The labeled microscope slide was hanged inside a 3000 ml plastic trough containing 20 ml of water (as a moisture chamber) to ensure that the relative humidity was near saturation. Room temperature was adjusted to 20°C using a 'carrier' air conditioner, and a thermometer was used to monitor the temperature in the moisture chamber. After 3 h, the pollen tubes had grown to a length three times the diameter of pollen grains. The percentage of pollen germination was recorded for 20 pollen grains from 6 microscope areas for each slide (Sharma and Sharma, 1972).

Statistical analysis

The data were subjected to analysis of variance (ANOVA), and Fisher's Least Significant Difference used to compare the means. Correlations were performed on the 28 genotype means to determine the relationship between pollen quantity, I_2KI-stained pollen frequency, and germination frequency.

Results and discussion

Quantity of pollen, the percentage of pollen grains stained with I_2KI, and the percentage of pollen grains that germinated on the media of nectar: water 1:9 (v/v) are shown in Table 1. The values of studied traits varied significantly ($P<0.05$) among the diploid banana hybrids. This indicates that there are inherent differences in the diploid banana hybrids in terms of pollen production and the conditions required for *in vitro* pollen tube germination.

The diploid banana hybrid with the highest rating for pollen quantity was '12506s-29' while the lowest rating for quantity was observed on the diploids '12506S-1' and '11025s-1'. The rating for pollen quantity was not significantly ($P<0.05$) different from the male fertile

Table 1. Mean relative pollen quantity, frequency of I_2KI-stained pollen, and pollen germination frequency in diploid banana hybrids

Genotype	Pedigree		Relative pollen quantity	I_2KI-stained pollen (%)	Pollen germination (%)
	Female	Male			
12506S-29	917K-2	7197-2	100.0*	88.0*	83.1*
OPP2-861	Opp1-861	-	95.0*	93.4*	88.0*
3162K-5	Fhia17	OPP1-861	90.0*	28.7	52.9
3162K-3	Fhia17	OPP1-861	87.5*	90.0*	82.2*
3162K-1	Fhia17	OPP1-861	87.5*	100.0*	65.8
3146K-3	7197-2	8075-7	75.0*	95.4*	81.7*
3146K-2	7197-2	8075-7	68.8*	97.4*	81.5*
12468S-1	917K-2	SH3217	64.0	56.9	48.1
3162K-2	FHIA17	OPP1-861	58.3	96.8*	87.0*
1537K-1	Kabucuragye	Cal4	57.5	50.7	39.6
12658S-1	917k-2	SH3362	57.3	74.2*	16.6
2866K-4	401K-1	SH3217	54.3	92.6*	88.2*
2858K-5	917K-2	8075-2	53.3	82.7*	1.7
8606S-1	222K-1	8075-7	50.0	53.0	32.2
3550K-1	OPP1-861	SH3362	50.0	77.6*	85.5*
2714K-7	222K-1	7197-2	43.8	50.0	53.9
12538S-1	917K-2	7197-2	40.0	43.6	27.2
12506S-3	917K-2	7197-2	33.3	34.4	29.5
12528S-12	1438K-1	SH3217	32.3	28.0	15.4
12591S-1	917K-2	SH3142	29.3	39.2	25.0
11025S-1	365K-1	7197-2	25.0	35.2	11.0
12506S-1	917K-2	7197-2	25.0	13.0	12.6
8075-7	SH3362	Cal4	66.8	88.5*	94.0*
861S-1	Nyamwezi	Cal4	57.3	65.2	65.1
7197-2	SH3362	Long tavoy	50.0	95.0*	93.0*
5105-1	Calcutta4	PsangLilin	45.0	57.8	56.0
OPP1-861	861S-1	-	45.0	90.0*	75.0*
Calcutt4	-	-	100.0	96.7	95.7
Mean			58.6	68.4	56.7
$LSD_{(0.05)}$			31.25	22.24	14.20

*not significantly different from the reference male parent Calcutta 4.

reference diploid 'Calcutta4' for seven diploid banana hybrids , namely'12506S-29', 'OPP2-861', '3162K-3', '3162K-1', '3162K-5', 3146K-3' and '3146K-2'.

The highest percentage of pollen grains that were stained blue-black with I_2KI were detected in the diploid banana hybrid '3162K-1' and the lowest in '12506S-1, which shows the differences in starch filling of the grains. The percentage of I_2KI-stained pollen grains was not significantly different ($P<0.05$) from 'Calcutta4' in 11 candidate diploid banana hybrids, namely '12506S-29','OPP2-861',

'3162K-3', '3162K-1', '3146K-3', '3146K-2', '3162k-2', '12658S-1', '286K-4', '2858k-5', and '3550K-1'.

The percentage of pollen grain germination was significantly correlated with pollen quantity and the percentage of pollen grains stained with I_2KI among the diploid banana hybrids (Figure 1).The highest pollen grain germination frequency, among the diploid banana hybrids was 81.2 % from '2866K' while the lowest germination frequency of 1.7% was from '2858K-5'.

The effectiveness of pollen fertility may be affected by pollen hydration, pollen germination, and pollen tube penetration through the stigma and style to the ovary (Shepherd, 1960; Wedzony and Filek, 1998). From these results, the significant correlation of pollen staining with pollen germination indicated that staining pollen with I_2KI and germinating pollen grains in nectar and water appear to be measuring similar aspects of the pollen grains' fertility. The I_2KI reagent distinguishes pollen viability on the basis of presence or

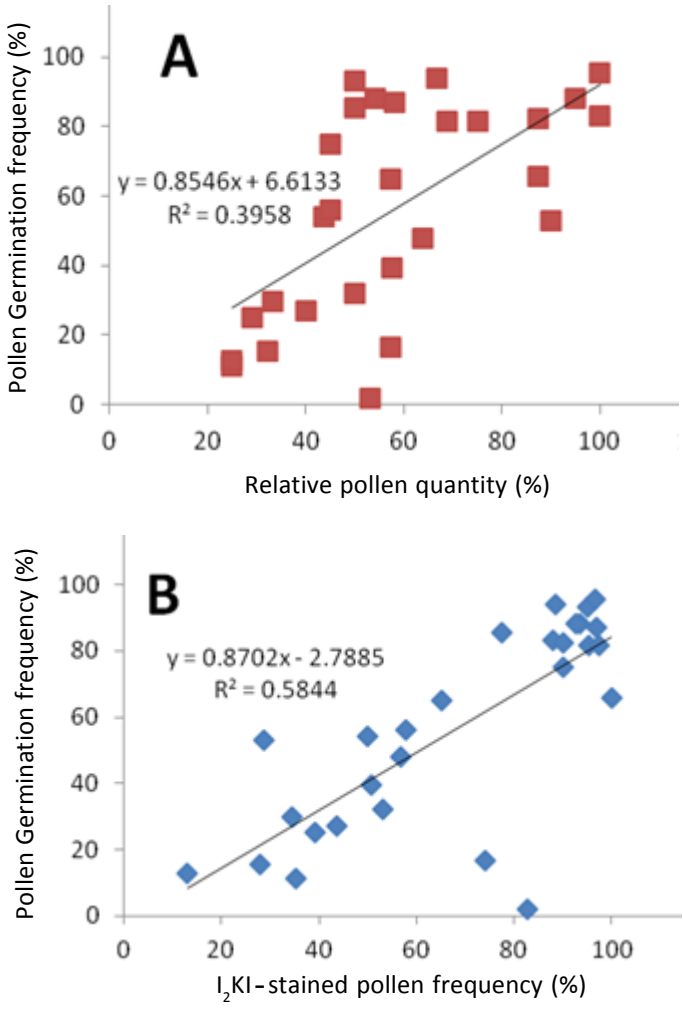

Figure 1. Correlation of pollen germination frequency with relative pollen quantity (A) and I_2KI-stained pollen frequency (B).

absence of starch granules. The presence of starch granules, which are storage molecules, in a pollen grain indicates availability of energy for germination and growth of the pollen tube during pollination and fertilization. This implies that pollen that fails to store starch granules are not considered functional. However, pollen grains that get impaired without affecting starch granules may erroneously still be considered functional. For instance, candidate diploid banana hybrids '2858K-5' and '12658S-1' showed high I_2KI-stained pollen frequency but low pollen germination frequency. Nyine and Pillay (2007) found similar results, emphasizing that pollen grain viability assessment through the staining method seems to express the pollen tube germination potential. Consequently, germination of the pollen is convincing indication of the actual fertility of the pollen grains (Sharma and Sharma, 1972). For practical reason, due to its low costs and simplicity, I_2KI staining of banana pollen grains can be recommended as a good estimation of pollen fertility due to its positive correlation with pollen tube germination rate that was confirmed by the present study.

All the banana diploids included in this study, that have previously been used to successfully generate progenies, had lower relative pollen quantity than the reference genotype 'Calcutta4'. However, 3 diploids, i.e. '8075-7', '7197-2', and 'OPP1861', were comparable in I_2KI-stained pollen and pollen germination frequencies to 'Calcutta4'. Despite lower pollen production than the reference genotype, the success of these banana diploids as male parents could have been due to the manipulations of the artificial pollination technique, such that more anthers were used for the parents producing less pollen.

In conclusion, pollen rating, I_2KI-stained pollen, and pollen germination frequencies were used to select five new diploid banana hybrids, i.e. '12506S-29', 'Opp2-861', '3162K-3', '3146K-3', and '3146K-2' for incorporation into banana breeding schemes at NARO. These diploids should be further evaluated for their response banana constraints like banana weevils, nematodes, and Fusarium wilt to assess their usefulness in breeding.

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