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Pollen Amount and Viability in Mchare and Selected Wild (AA) Banana (*Musa acuminata*) Genotypes: Prospects for Breeding

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Abstract. **East African diploid cooking bananas, commonly called Mchare, are a staple crop for millions of subsistence farmers in Tanzania, particularly in the Pangani region in northern Tanzania. Several pathogens constrain Mchare production significantly and threaten food security. Sources of resistance to these pathogens have been identified; however, partial male and female sterility impedes successful resistance introgression, complicating the breeding process. Mchare cultivars are also the only known surviving representatives of a diploid banana subgroup that contributed unreduced gametes to many of the most widely grown and successful triploid dessert bananas ('Cavendish', 'Gros-Michel', 'Silk', and 'Prata'). As such, they represent an essential intermediate step in the conventional improvement of bananas worldwide. We assess the amount and viability of pollen among Mchare and wild genotypes to identify the most fertile Mchare cultivars that can be used in conventional banana improvement. Pollen was collected from 14 banana genotypes for quantification and viability testing over 7 months, and the optimal time for pollen collection was determined to be 0800 HR. Significant variation among banana genotypes in terms of both overall pollen production and percentage of pollen viability was observed. The wild-type bananas 'Calcutta 4' [International Musa Germplasm Transit Center (ITC) 0249] and 'Borneo' (ITC0253) had the greatest overall pollen production (> 31,000 pollen grains/anther) and viability (~74%), whereas 'Ijihu Inkundu' (ITC1460; Mchare genotype) was the least productive (almost completely sterile), with an average pollen production of a few hundred grains per anther and a viability of 7%. There were significant differences among months in terms of pollen viability, with the greatest average viability observed in May, April, and February (> 51%), and the lowest average pollen viability in July (41%). Significant differences were observed among the Mchare genotypes, with 'Huti-White', 'Huti green bell' (ITC1559), and 'Mchare Laini' consistently producing more substantial amounts of total pollen and an overall more significant proportion of viable pollen. This information is vital to improve Mchare bananas and the global breeding of dessert bananas. The choice of Mchare banana used in improvement programs could affect fertility and the likelihood of breeding success.**

Bananas are one of the world's most important fruit crops and are among the 10 most important staple crops (Petsakos et al. 2019). They are also an important source of income for millions of people in tropical and subtropical regions of the world, and have particular importance in the Great Lakes region of Africa (Ndabamenye et al. 2013; Nyine and Pillay 2007; Taulya 2013). East African Highland cooking bananas are a daily staple for more than 20 million people in the region (Meya et al. 2023). In Tanzania, bananas are an essential food and commercial crop, and a significant source of raw materials for the beverage and handcraft industries (Luzi-Kihupi et al. 2015). Despite their recognized importance for food security, banana production is low due as a result of the susceptibility of current genotypes (landraces) to insects, nematodes, and diseases (Nyine and Pillay 2007), and low nutrient supplies (Amah et al. 2020; Meya et al. 2023). These constraints include black Sigatoka (*Mycosphaerella fijiensis*), Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*), and bacterial wilt (*Xanthomonas* spp.) (Okole et al. 2000; Vishnevetsky et al. 2011). Viral diseases include Banana streak virus and banana bunchy top (Jain and Priyadarshan 2009; Perrier et al. 2011; Shimwela et al. 2022). These bananas are also susceptible to pests such as weevils and nematodes (Ssali et al. 2012; Ssebuliba et al. 2008). The decline in production has led to food security issues, particularly for farmers in rural areas who depend on bananas as their principal source of carbohydrates and an essential source of supplemental income (Nyine and Pillay 2007).

Since the 1920s, research centers have been engaged in the hybridization-based breeding of bananas (Jain and Priyadarshan 2009). The EMBRAPA-CNPMF in Brazil, the NRCB, and the TNAU in India all strive to breed indigenous varieties of dessert and cooking bananas, whereas the FHIA in Honduras breeds bananas for export as well as "cooking" varieties. The CARBAP in Cameroon and the IITA are conducting research regarding the breeding of plantains and bananas in Africa (Jain and Priyadarshan 2009; Ortiz et al. 1995). Most cultivated bananas are triploids ($2n = 3x = 33$), which complicates breeding efforts and requires intensive resource allocation to develop superior varieties (Brown et al. 2017; Ortiz et al. 1995). The initial steps in conventional crossbreeding of bananas are hybridizing and selecting superior diploid recombinants to introduce desired traits (Aguilar Morán 2013; Ortiz and Vuylsteke 1996). Improved, disease-resistant, and pollen-fertile diploids are subsequently crossed with preferred triploid ($3x$) varieties to produce tetraploid plants ($4x$) that, in turn, are crossed by improved diploids in a second cycle to produce secondary triploids (Adeleke et al. 2002). With this crossing scheme, poor seed set is a bottleneck, with fertility varying considerably among genotypes and cultivars. Environmental factors influence fertility, including time of the year, and variations in moisture, humidity, daylength, and temperature (Brown et al. 2017; Ortiz and Vuylsteke 1995). Weather conditions, such as high temperatures, solar

radiation, low rainfall, and high evapotranspiration, reduce the size of the bunch and fruit (Waniale et al. 2021). This can affect seed set in *Musa* spp. Because there is a correlation between reduced fruit circumference of 'Gros Michel' and reduced seed set, which may indicate a reduction in parthenocarpy (Dzoyem et al. 2024; Shepherd 1954). Increased seed set in Matooke correlates with high temperatures, solar radiation, and low rainfall (Ssebuliba et al. 2009). The environmental factor impact on seed set is poorly understood, but distinct seasonal effects have been noted (Ortiz and Crouch 1997; Ortiz and Vuylsteke 1995). Although there are a considerable number of genetic and cytogenetic factors (i.e., parthenocarpy, low seed viability, irregular meiotic behavior, and diverse genomic configurations) that likely also contribute to poor seed set in bananas (Amah et al. 2020; Batte et al. 2019; Němečková et al. 2018; Ortiz and Swennen 2014; Waniale et al. 2021), limited pollen viability has been noted as a critical limitation.

Many wild (seeded) diploid bananas produce abundant pollen, which generally have a greater amount of viable pollen when compared with cultivated (seedless) bananas (Fortescue and Turner 2004). The quantity and viability of pollen are important considerations when selecting male parents (Ssebuliba et al. 2008) and are indispensable in the efficient genetic improvement of bananas (Fortescue and Turner 2005). Wild diploid bananas such as 'Calcutta 4' [International Musa Germplasm Transit Center (ITC) 0249] and 'Borneo (ITC0253) produce abundant pollen and have been used extensively as donors of disease resistance alleles by multiple breeding programs (Pillay et al. 2012). Unfortunately, they also contribute unfavorably to several important agronomic traits such as small bunch and fruit size, and reduced shelf life and consumer acceptance. Mchare (also called Mlali or Muraru) bananas, grown extensively in certain highland regions of East Africa (and some East African islands) as cooking bananas, are currently being bred for improvement for multiple disease resistance, including *Fusarium* (race 1 and Foc TR4), which threatens banana production worldwide (Brown et al. 2017). Mchare is recognized as the unreduced gamete source for some of the most economically important dessert bananas globally, including 'Cavendish', 'Gros Michel', 'Pome or Prata', and 'Silk' bananas, contributing two-thirds of the genomic complement to each of these triploid dessert bananas types (Jeensae et al. 2021; Jeridi et al. 2012; Martin et al. 2020; Perrier et al. 2011; Raboin et al. 2005). Mchare (formerly Mlali) subgroup was revealed as the closest 2*n* gamete donor for the 'Cavendish' and 'Gros Michel' subgroups (Jeensae et al. 2021; Martin et al. 2017, 2020; Perrier et al. 2011). Thus, improved diploid Mchare could efficiently introduce disease resistance into related and economically important dessert bananas. Therefore, the improvement of these bananas not only has immediate importance to food security in Africa, but also holds promise as an essential donor source in the conventional improvement of dessert bananas worldwide.

Despite the importance of these cultivated (parthenocarpic) diploid (2*n*) bananas, more information is needed regarding pollen amount and viability among Mchare genotypes. A single Mchare (syn. Mlali) cultivar, Chicame, from the Comoros islands has 40% pollen viability in the diploid state, and after chromosome doubling for use as a tetraploid in the breeding scheme, the percentage of viable pollen was observed to increase to 61.3% (Goigoux et al. 2013).

In our study, we focused on the differences in amount and viability of pollen over 7 months (covering the dry and rainy seasons in East Africa) to determine whether there are significant differences among Mchare genotypes that can be exploited for greater breeding efficiency (Stone et al. 1995).

Materials and Methods

We evaluated 14 diploid banana genotypes as mature plants in the second production cycle (first ratoon) planted at the International Institute of Tropical Agriculture (IITA) banana fields at the World Vegetable Center in Arusha, Tanzania (lat. 3°22′1″S, long. 3°22′11″E, elevation 1264 m). Seven diploid banana accessions represent known variability and consumer preference among cultivated Mchare in Tanzania. Five accessions were included for comparison, including wild-type banana diploids used as donor sources for disease resistance and two cultivated (seedless) diploids (Table 1). All genotypes are AA diploids. Plants within blocks were spaced at 3 × 2 m and had been planted previously in a randomized complete block design with three replications. All plants were rainfed, with a maximum rainfall of 289 mm observed in April and a minimum of 11 mm observed in July; the average rainfall per month for 2017 was 88 mm, the average maximum temperature was 28 °C in February, and the average minimum temperature was 13 °C in July (Fig. 1). Data were collected once a month for 7 months (from Feb to Aug 2017).

Pollen collection. To determine the optimal time for pollen collection, six diploids ['Calcutta 4' (ITC0249), 'Borneo' (ITC0253), 'Huti-White', 'Pisang Lilin' (ITC1121), 'Zebrina G.F.' (ITC0966), and 'Akondro mainty' (ITC0281)] were assayed for amount and viability in 2-h intervals from 0600 to 1400 HR once a week for 4 weeks. Based on the results, pollen collection was standardized at 0800 HR for all subsequent assays.

Table 1. Fourteen banana genotypes used in pollen variation and viability studies.

No.	ITC code[i]	Cultivar	Subspecies	Type
1	ITC0249	Calcutta 4	*burmannica*	Wild
2	ITC0253	Borneo	*microcarpa*	Wild
3	ITC0712	CV Rose	*malaccensis*	Cultivated
4	ITC1121	Pisang Lilin	*malaccensis*	Cultivated
5	ITC0609	Pahang	*malaccensis*	Wild
6	ITC0393	Truncata	*truncata*	Wild
7	ITC0966	Zebrina (G.F.)	zebrina	Wild
8	ITC1559	Huti green bell	Mchare	Cultivated
9	—	Huti-White	Mchare	Cultivated
10	—	Mchare laini	Mchare	Cultivated
11	ITC1446	Makyughu-II	Mchare	Cultivated
12	ITC1455	Mchare mlelembo	Mchare	Cultivated
13	ITC0281	Akondro mainty	Mchare	Cultivated
14	ITC1460	Ijihu Inkundu	Mchare	Cultivated

[i] ITC = International Musa Germplasm Transit Center.

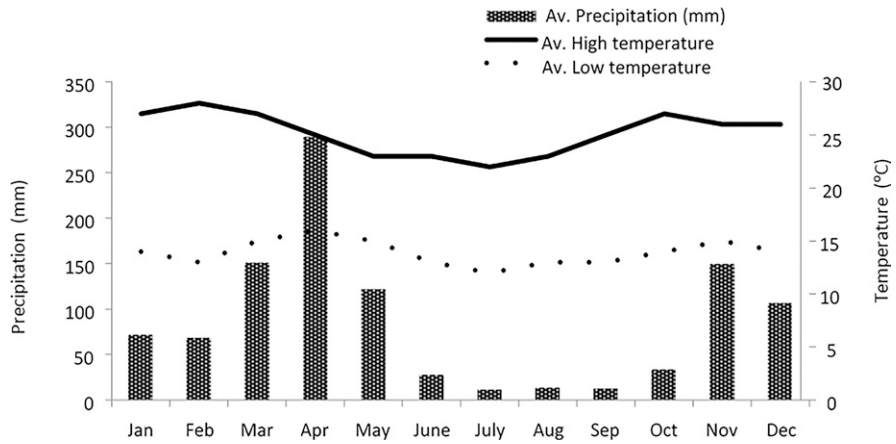

Fig. 1. Average rainfall and temperature in Arusha, 2017.

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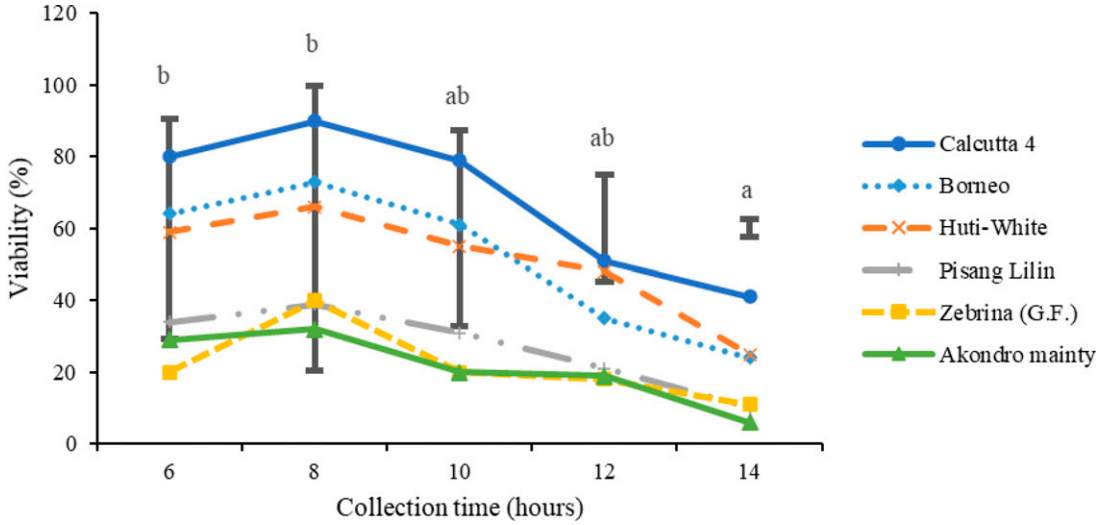

Fig. 2. Average pollen viability of six selected banana genotypes collected at different times of the day. Bar = least significant difference. Means with a similar letter are not significantly different at $P \leq 0.05$.

Quantification of pollen grain. Because bananas flower in a nonseasonal manner, plants were selected based on availability. The third hand of emerging male flowers (subsequent clusters of flowers emerge each day) was sampled, and pollen was collected from three anthers of three plants per genotype. Plants were selected randomly. Pollen was removed carefully from the anthers with sterilized tweezers and distributed evenly on microscope slides. One drop of detergent solution (prepared by diluting two drops of commercial dishwashing soap in 250 mL distilled water) was added to the sample to disperse the pollen grains. A Nikon SM275T Zoom stereomicroscope with ×6.5 to ×22.5 magnification (Nikon, Tokyo, Japan). The attached camera was used to take digital images (JPEG format) that were analyzed with Image J software (Rasband 2008) to provide counts of pollen grains (Costa and Yang 2009). Each image was processed individually to sharpen it, remove noise, and enhance the individual pollen grains to achieve and accurate count. We did not checked for haploidy or unreduced pollen, and we did not measure pollen size.

Table 2. Analysis of variance for pollen production and viability for 7 months in 2017.

Source of variation[i]	df	Pollen production sum of squares	Pollen production mean square[ii]	Pollen viability sum of squares	Pollen viability mean square[ii]
Replication	2	89,920,000	44,960,000^{NS}	125.2	62.6^{NS}
Month	6	213,420,000	35,570,000^{NS}	4,460.16	743.36***
Cultivar	13	38,103,000,000	2,931,000,000***	91,750.1	7,057.7***
Month × cultivar	76	2,122,680,000	27,930,000^{NS}	7,790.76	102.51***
Residual	190	4,142,000,000	21,800,000	5,891.9	31.01
SD	—	—	4,669.05	—	5.57
CV (%)	—	—	30.3	—	11.7

[i] *CV* = coefficient of variation; *SD* = standard deviation.
[ii] NS, *** Nonsignificant or significant at $P \leq 0.05$, respectively.

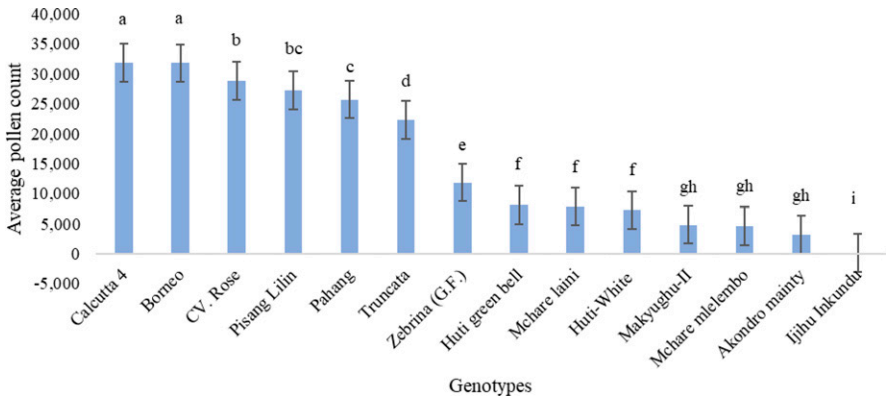

Fig. 3. Average amount of pollen per anther of 14 diploid banana genotypes observed for 7 months in 2017. Means with a similar letter are not significantly different at $P \leq 0.05$. The least significant difference value for genotypes is 1715.47.

Pollen viability test. Pollen viability of 14 banana genotypes was assayed according to Soares et al. (2016). Pollen grains were stained with a 1% triphenyl tetrazolium chloride (TTC) solution diluted in Tris buffer (0.15 M HCl, pH 7.8), followed by a 2-h incubation period at room temperature. Four subsample counts were carried out from multiple regions of the slide (25 grains per region) using a stereomicroscope. Viable pollen grains stained by TTC appear light to dark red, whereas nonviable pollen grains remain translucent in the presence of the stain (Soares et al. 2016). The number of viable and nonviable pollen grains was converted to a percentage using (Viable pollen/Total pollen) × 100.

Statistical analysis. An analysis of variance (ANOVA) was conducted using GenStat software (21st edition, 64-bit) ($P \leq 0.05$). The linear model used was $Y_{ijkl} = \bar{\bar{Y}} + G_i + R_j + M_k + GM_{ik} + e_{ijkl}$, where $\bar{\bar{Y}}$ is the grand mean, G_i is the genotype mean effect, R_j is the replication effect, M is the month effect, GM is the interaction between the genotype effect and the month effect, and e_{ijkl} is the experimental error. All effects were assumed to be fixed except for month. Mean separation was accomplished using Fisher's protected least significant difference (LSD) at a 5% probability level if the F value was significant. In addition, a Pearson correlation of pollen viability and amount (pollen grains per anther) was also conducted with the same software.

Results

Our results show that pollen viability peaked at 0800 HR for all six cultivars and declined dramatically thereafter (Fig. 2). Lower viability was observed during earlier and later observations, and this was independent of the banana genotype. An ANOVA of total pollen production revealed significant differences ($P \leq 0.05$) among the genotypes (Table 2). Average pollen production over the 7 months ranged from more than 31,000 grains/anther to

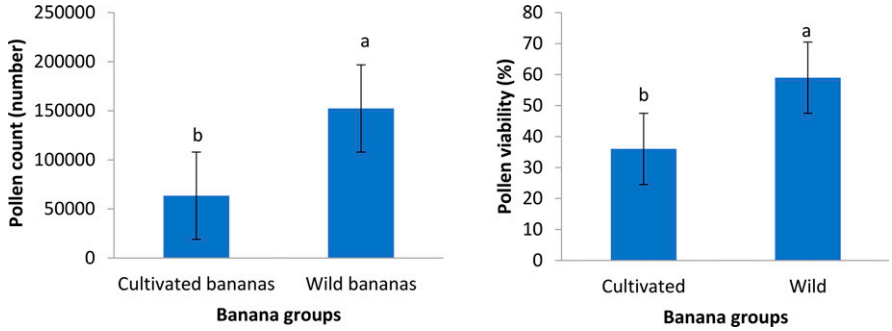

Fig. 4. Comparison of pollen production and viability between cultivated and wild banana genotypes. Bar = least significant difference. Means with a similar letter are not significantly different at $P \leq 0.05$.

155 grains/anther, with 'Calcutta 4' and 'Borneo' producing more than 31,000 grains/anther, followed by 'CV Rose' (28,847 grains/anther), 'Pisang Lilin' (27,262 grains/anther), 'Pahang' (25,715 grains/anther), and 'Truncata' (22,259 grains/anther), which was significantly less than 'Calcutta 4' and 'Borneo' (Fig. 3). The Mchare genotypes produced significantly less pollen than the wild-type bananas (Fig. 4), but there were significant differences among the Mchare genotypes. 'Huti green bell' (8120 grains/anther) and 'Mchare laini' (7875 grains/anther) represented ~20% of the pollen observed in 'Calcutta 4', whereas others ('Mchare mlelembo' and 'Akondro mainty') produced ≈10%. 'Ijihu Inkundu' was almost completely sterile, producing either no pollen or only trace amounts, depending on the month (Table 3). Our results showed no significant difference among months in number of pollen delivered (Table 3). However, the trend suggested that cultivars produced greater amounts of pollen during the annual rainy season, which spans from April (16,501 grains/anther) to June (16,685 grains/anther); the lowest average pollen counts occurred during the driest month (July; 14,451 grains/anther).

An ANOVA for pollen viability revealed significant differences among banana genotypes ($P \leq 0.05$) (Table 2). 'Borneo' and 'Calcutta 4' recorded the greatest amount of viable pollen (74.2%) and were not significantly different from each other (Fig. 5), but were very different from 'CV Rose' (64.6%), 'Huti-White' (59.3%), and 'Pahang' (57.2%). The lowest pollen viability was observed in the Mchare cultivar Ijihu Inkundu (7.3%) (Table 4).

There was a significant difference in terms of average pollen viability over the 7 months, suggesting that pollen viability was influenced by factors associated with the environment (Table 2). The highest amount of viable pollen (51%) was observed during rainy months (May and April), and the lowest average pollen viability was observed in July (41%), which is typically a drier and cooler month (Table 4). There was a significant interaction between month and genotype in pollen viability, suggesting that genotypes may behave differently (Table 2). A positive correlation was observed between the amount of pollen produced per anther and the percentage of pollen viability ($r = 0.76$), but exceptions were noted (Fig. 6).

Discussion

Pollen viability. Maximum pollen viability in banana genotypes was observed when pollen was collected at 0800 HR, regardless of the genotype (Fig. 2). Similar results were reported by Kaefer et al. (2016), who collected pollen from maize at different time points (0900, 1400, and 1600 HR) and determined that pollen collected in the morning presented a greater germination rate. Mondo et al. (2022) reported that the pollination success rate in yams (*Dioscorea alata*) is more conducive in the morning (0800–1200 HR), although *Dioscorea rotundata* was more conducive in the afternoon (1200–1700 HR). They concluded that midday hours were more appropriate for crossing. The results are also supported by Soares et al. (2015), who postulated that banana pollination success should be high in the morning (0700–1000 HR) as a result of maximal pollen viability and stigma receptivity. High pollen viability in the morning could be associated with lower temperatures and/or a lower vapor pressure deficit, and some have observed that higher temperatures harm pollen viability (Ortiz 2011; Rang et al. 2011; Wang et al. 2004). On the contrary, Brunet et al. (2019) did not observe a temperature effect on pollen viability in alfalfa (*Medicago sativa*). Low pollen viability could also be attributed to additional environmental factors, and further work is required to understand the phenomena more completely (Johri and Vasil 1961), but it has been suggested that bananas can be highly affected by slight changes in environmental conditions (Ortiz et al. 1998; Stone et al. 1995; Turner et al. 2007).

Amount of pollen grains. Our work showed that the amount of pollen grains per anther varied significantly among banana genotypes, with wild, seeded banana genotypes producing the greatest amount of pollen (with greater viability) than cultivated bananas. Similar results were reported by Soares et al. (2016). According to Tenkouano et al. (1998), the wild diploid ('Calcutta 4') produces ≈10% higher pollen rates when compared with cultivated diploids. Low pollen production among banana cultivars can hinder banana improvement because the production of viable seeds through hybridization is extremely limited and critical for breeding success. It can take several months to obtain seeds from a desired cross, and the production of seed is generally poor and has been reported to be 0.3 to 21.7 seeds per bunch

Table 3. Average amount of pollen per anther of 14 diploid banana cultivars for 7 months in 2017.

Cultivar	Feb	Mar	Apr	May	Jun	Jul	Aug	Total	Mean[i]	*SD*[ii]
Calcutta 4	33,550	31,217	31,023	32,793	32,176	30,784	31,499	223,042	31,863 a	1,018.9
Borneo	32,779	34,510	33,456	29,351	29,483	30,905	32,158	222,642	31,806 a	1,972.3
CV Rose	29,206	30,162	29,309	28,158	30,037	25,346	29,714	201,932	28,847 b	1,681.8
Pisang Lilin	27,167	29,343	27,444	27,040	26,899	26,193	26,749	190,835	27,262 bc	996.6
Pahang	31,807	28,082	29,726	22,321	17,535	26,264	24,266	180,001	25,715 c	4,824.2
Truncata	25,494	23,669	23,697	21,556	21,455	21,561	18,378	155,810	22,259 d	2,280.8
Zebrina (G.F.)	8,699	12,728	16,513	9,470	11,234	11,751	12,450	82,845	11,835 e	2,544.8
Huti green bell	4,211	3,939	8,933	5,864	22,402	5,722	5,769	56,840	8,120 f	6,503.4
Mchare laini	5,046	5,010	6,474	5,667	22,306	5,107	5,514	55,124	7,875 f	6,384.1
Huti-White	5,840	7,022	9,920	6,900	6,662	6,591	7,876	50,811	7,259 f	1,320.5
Makyughu-II	4,748	4,577	5,794	5,236	4,877	4,472	4,194	33,898	4,842 g	532.1
Mchare mlelembo	2,676	5,106	5,168	5,497	5,059	4,477	4,006	31,989	4,570 g	971
Akondro mainty	3,028	3,347	3,369	3,219	3,245	2,913	3,068	22,189	3,170 g	171.1
Ijihu Inkundu	0	0	197	199	222	232	237	1.087	155 h	107.2
Total	214,251	218,712	231,023	203,271	233,592	202,318	205,878	—	—	—
Monthly mean	15,304 bc	15,622 a–c	16,502 ab	14,519 c	16,685 a	14,451 c	14,706 c	—	—	—

[i] Means with a similar letter in the same column and same row are not significantly different at $P \leq 0.05$. The least significant difference value for cultivars is 1715.47. The least significant difference value for the monthly mean is 9437.97.
[ii] *SD* = standard deviation.

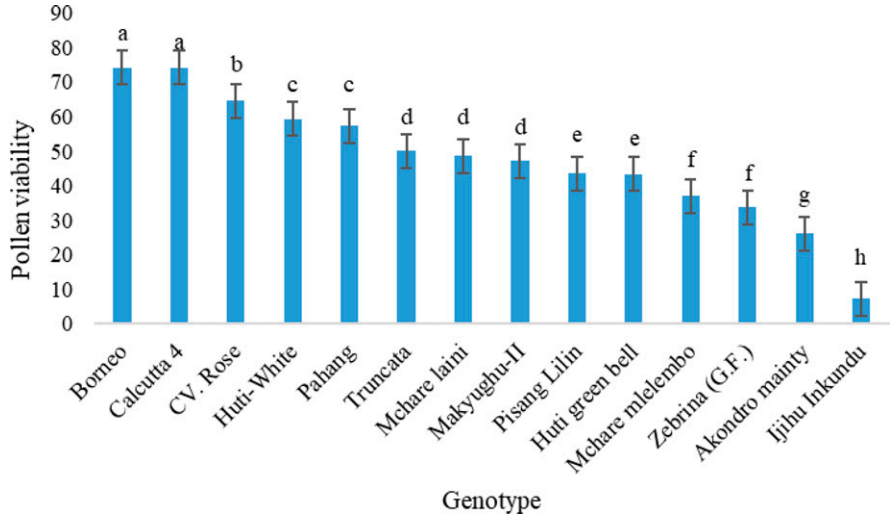

Fig. 5. Average pollen viability per anther of 14 diploid banana genotypes for 7 months in 2017. Means with a similar letter are not significantly different at $P \leq 0.05$. The least significant difference value for genotypes was 3.71.

(Batte et al. 2019; Vuylsteke et al. 1993). It is interesting to note that the *malaccensis* subspecies cultivars CV Rose and Pahang in our study produced significantly less pollen than the representatives of the subspecies *burmannica* ('Calcutta 4') and *microcarpa* ('Borneo'). Further work is required to determine whether these results are characteristics of the subspecies.

Our results confirm that wild bananas produce greater amounts of pollen than cultivated bananas (Fig. 4). This could be caused by chromosomal heterogeneity in many important cultivated bananas (including Mchare), as they have been demonstrated to be hybrids of two or more subspecies (Martin et al. 2017).

Pollen viability. Pollen viability percentage was considerably greater in wild-type bananas compared with cultivated bananas (Fig. 4). Adeleke et al. (2004), and Dumpe and Ortiz (1996) in their fertility studies in diploids and triploids, found that the wild banana 'Calcutta 4' had very high pollen viabil-

Table 4. Average pollen viability per anther of 14 diploid banana cultivars for 7 months in 2017.

Cultivar	Feb	Mar	Apr	May	Jun	Jul	Aug	Total	Mean[i]	*SD*[ii]
Borneo	79.1	84.3	77.4	77.1	67.7	64.8	68.8	519.2	74.2 a	7.1
Calcutta 4	79.1	84.3	77.4	77.1	67.7	64.8	68.8	519.2	74.2 a	7.1
CV Rose	75.6	67.0	73.4	61.3	56.9	58.3	59.4	452.0	64.6 b	7.5
Huti-White	64.3	61.6	62.1	65.5	56.5	49.2	55.9	415.2	59.3 c	5.8
Pisang Pahang	55.2	52.0	67.4	67.4	59.1	49.2	49.7	400.1	57.2 c	7.8
Truncata	66.8	63.9	47.9	56.4	38.9	35.5	40.6	349.9	50 d	12.5
Mchare laini	58.9	55.1	47.7	56.1	43.9	38.5	39.1	339.4	48.5 d	8.4
Makyughu-II	50.6	52.3	53.7	51	42.2	42.2	38.4	330.4	47.2 d	6.1
Pisang Lilin	46.8	36.0	48.9	46.9	45.4	36.0	44.2	304.4	43.5 e	5.3
Huti green bell	37.8	36.2	52.7	48.2	47.2	39.2	42.1	303.4	43.3 e	6.2
Mchare mlelembo	41.3	41.5	36.4	41.2	35.8	29.2	32.6	258.0	36.9 f	4.8
Zebrina (G.F.)	26.4	27.9	29.4	37.9	38.5	37.6	39.1	236.7	33.8 f	5.6
Akondro mainty	31.5	26.1	29.8	26.1	27.5	20.6	20.8	182.5	26.1 g	4.2
Ijihu Inkundu	0	0	11.9	10.2	8.1	10.2	10.1	50.6	7.2 h	5.1
Total	713.6	688.2	716.2	722.4	635.6	575.1	609.6	—	—	—
Monthly mean[i]	51.0 ab	49.2 b	51.2 ab	51.6 a	45.4 c	41.1 d	43.6 c	—	—	—

[i] Means with a similar letter in the same column and same row are not significantly different at $P \leq 0.05$. The least significant difference value for cultivars is 3.71. The least significant difference value for the monthly mean is 14.75.
[ii] *SD* = standard deviation.

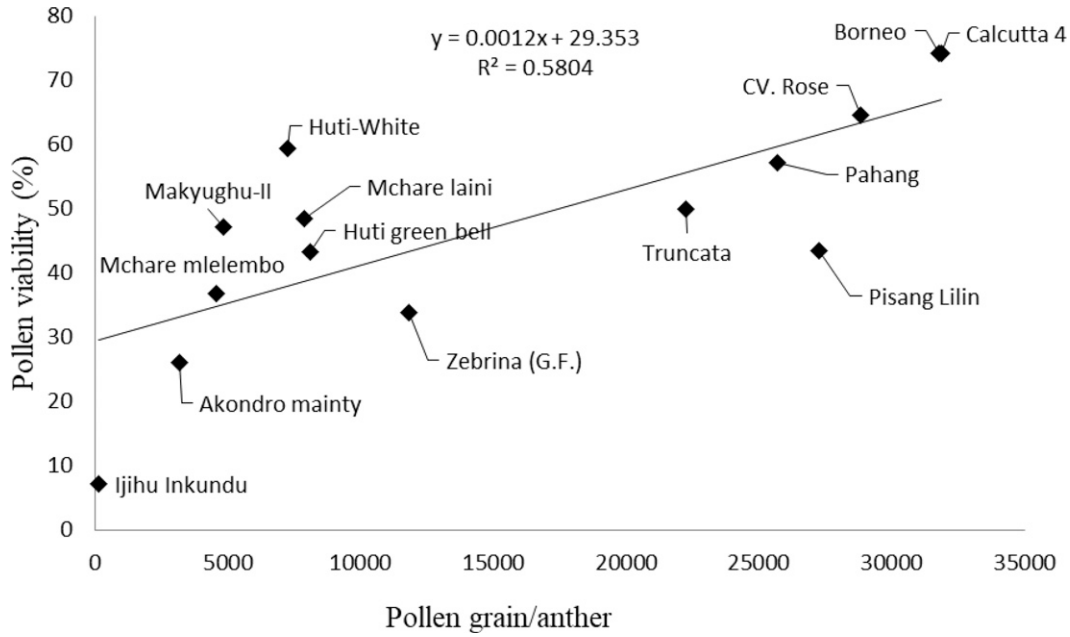

Fig. 6. Association between amount of pollen and viability in 14 banana genotypes.

ity compared with other diploids. Dumpe and Ortiz (1996) estimated that 'Calcutta 4' and 'Borneo' had pollen viability in Nigeria up to 98%, based on staining ability. Although the estimates of these two genotypes conducted in Tanzania were less than reported previously in Nigeria, where humidity is much higher, they outperformed all other banana genotypes observed.

Effects associated with month were observed to affect pollen viability among banana genotypes. We observed the highest pollen viability from February to May, with greater precipitation and ambient temperature, and the lowest pollen viability in July, which combines a dry period with the lowest ambient temperature. Similar results by Ssebuliba et al. (2009) were observed regarding seed set, with two major seed set peaks occurring in March and April, with a minor peak in September. In our study, pollinations were done from February to August. Based on meteorological data, the maximum temperature was recorded in February (28 °C), followed by March (27 °C), then April (25 °C), whereas the minimum temperature was recorded in July (12 °C). March, April, May, and November had the greatest precipitation: 151, 289, 122, and 149 mm, respectively (Fig. 1). Tenkouano et al. (1998) observed that greater pollen viability in Nigeria was associated p associated with high solar radiation and higher temperatures, and was associated negatively with high rainfall and high relative humidity.

The Mchare subgroup of bananas has been recognized as the unreduced gamete donor of some of the most important triploid dessert bananas consumed worldwide ('Cavendish', 'Gros Michel', 'Pome', and 'Silk' subgroups) (Jeensae et al. 2021; Martin et al. 2020, 2023; Perrier et al. 2011). The improvement of these triploid bananas with wild germplasm has proved problematic because of lack of good sensory traits in the offspring (Raboin et al. 2005). Fortunately, some of the greatest pollen-producing Mchare genotypes [also with the greatest percentages of viable pollen (i.e., 'Huti-White' and 'Mchare laini')] have farmers preferred characteristics. Although the published data in this area are limited, some of these genotypes ('Mchare laini' and 'Mchare mlelembo') are in greatest demand in Tanzanian markets. To progress in any recurrent breeding strategy, male and female fertility is a prerequisite, as genetic improvement of bananas is hampered by male and female sterility in cultivated genotypes (Ssebuliba et al. 2009). The observed differences in pollen viability among the cultivated Mchare bananas suggest that choices can be made between the different Mchare genotypes.

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