



Research Article

Screening for male fertility status in selected banana genotypes

Asish Kumar Panda, K. Soorianathasundaram* and R. M. Vijayakumar

Department of Fruit Crops, Horticultural College and Research Institute,
Tamil Nadu Agricultural University, Coimbatore, 641003 (India)

*E-Mail: sooria@tnau.ac.in

(Received: 22 Aug 2019; Revised: 24 Sep 2019; Accepted: 25 Sep 2019)

Abstract

In the present study, twenty-five banana cultivars (eight diploids and seventeen triploids) were screened for their male fertility status with reference to the pollen output per anther, pollen size and pollen viability. The results showed that the pollen output and pollen viability are higher in monospecific diploids than the cultivars having the genome of both *Musa acuminata* and *Musa balbisiana*. Pollen size were higher in triploids than the diploids. The cultivars such as 'Rose', 'Hatidat', 'Athiakol', 'Bhimkol' and 'Popoul' were reported to be polleniferous similar to proven male fertile parents such as 'Pisang Lilin' or 'Anaikomban'. The complexities arising out of ploidy status and genomic differences not only impact pollen viability but also pollen production.

Key words

Banana, Palynology, Nectar media, Multivariate analysis

Introduction

Most cultivated bananas are triploids ($2n=3x=33$) and some diploid cultivated varieties ($2n=2x=22$) also exist. As reported by Simmonds and Shepherd (1955), most of the cultivated bananas (*Musa* spp.) owes their origin to the natural crossing of its wild progenitors, predominantly *Musa acuminata* ($2n=2x=22$, AA genome) and *Musa balbisiana* ($2n=2x=22$, BB genome). The intrinsic variability available in the crop could be attributed to the involvement of several sub species and natural mutations and as well varied ploidy status. Among the biotic constraints limiting banana production, the prevalence of Fusarial wilt, Sigatoka leaf spot, pathogenic nematodes and viruses are considered most important. Resistant breeding programme in banana could not be successfully accomplished due to several reproductive complexities such as poor male and female fertility, vegetative parthenocarpy, presence of polyploidy and structural aberration of various sex organs. Conventional breeding strategies require parents with desirable attributes and as well high female or male fertility. The expression and regulation of various sterility factors governing the male as well as female sterility largely depends on the genomic constitution of the banana genotypes and is an integrative function of the interaction of differential contribution from the A and B genome. In a few instances, there are also evidences supporting variation in intra-genomic group level (Cenci *et al.*, 2019). Since the extent of fertility varies among banana cultivars, screening for male fertility attributes is important to identify promising donor

pollen parents which could be helpful in planning the hybridization programme. In the present study, an attempt has been made to study the palynological aspects related to male fertility of 25 select genotypes of banana maintained in the field gene bank of Tamil Nadu Agricultural University, Coimbatore.

Materials and Methods

The present study was taken up with eight diploid and 17 triploid banana cultivars which include 'Rose' (AA), 'Pisang Lilin' (AA), 'Anaikomban' (AA), 'Hatidat' (AA), 'Sannachenkadali' (AA), 'Athiakol' (BB), 'Bhimkol' (BB), 'Ney Poovan' (AB), 'Nendran' (AAB), 'Quintal Nendran' (AAB), 'Popoul' (AAB), 'Red Banana' (AAA), 'Grand Naine' (AAA), 'Rasthali' (AAB), 'Hill Banana' (AAB), 'Karpooravali' (ABB), 'Monthan' (ABB), 'Bangrier' (ABB), 'Chakkiya' (ABB), 'Klue Numkhom' (ABB), 'H-201' (AB) a synthetic diploid hybrid of TNAU and four pre-release cultures of TNAU viz., 'H-531' (AAB), 'H-916' (AAB), 'H-212' (AB), 'NPH-02-01' (AAB). Among these, Pisang Lilin and Anaikomban are known to possess higher male fertility (Sathiamoorthy, 1994) but was included in the study for comparison of male fertility status of other genotypes.

For pollen studies, the functionally male florets were collected the cool hours of early morning between 7.00 to 9.00 am by manually lifting the yet-to-open tenth bract of the male phase. The pollen was gently squeezed out and collected in the

laboratory by using a stainless-steel needle and camel hair brush. The parameters on pollen output, pollen size, pollen stainability and pollen germinability were studied as described below.:

Pollen output : The quantification of pollen produced per anther was done following the hemocytometry protocol (Sathiamoorthy, 1973). The pollen from all the five anthers of the male floret was squeezed out and dissolved in one ml of distilled water with a drop of soap solution to make the pollen properly dispersed. Pollens from separate male florets were taken as separate replications. Using a Pasteur pipette, single drop of the dispersed pollen suspension was carefully placed on one side of the haemocytometer channel without disturbing the pre-placed clear cover slip. The individual pollen grains from each of the quadrant of haemocytometer were counted and denoted as Q_{1-4} . The dilution factor (DF) was used for estimating the final pollen count using the following equation.

Pollen output per anther

$$= \frac{Q_1 + Q_2 + Q_3 + Q_4}{4 \times \text{Total number of anthers taken}} \times \text{DF} \times 1000$$

Pollen size: The diameter of pollen was measured as the representative pollen size under 100X magnification. The pollen diameter was measured using a mounted digital camera on an optical microscope and the size measurements were done after standardized image capture using image processing software Biowizard®. For each genotype, 100 pollen grains were measured, the mean worked out and expressed in microns (μ).

Pollen Stainability: To study pollen stainability, the pollen grains obtained were placed on a glass slide. A drop of one percent acetocarmine was placed on the pollen. The number of pollen grains that took the red colour stain was counted for 100 pollen grains and the percentage calculated.

Pollen germinability (*in vitro*) : The pollen grains collected from the fresh anthers were placed on a cavity slide. The banana nectar collected from 'Pisang Lilin' (AA) and diluted 1:9 was used as the pollen germination media using the protocol described by Nyine and Pillai (2007). Pollen germination counts were made within 24 hours after placing them on the media at a room temperature of $28 \pm 1^\circ\text{C}$

Multivariate Analysis: The mean and standard errors of the data set were calculated and correlation and multivariate analyses were done

using R-package of the RStudio version © 12.3.1335 (R Core Team, 2019).

Results and Discussion

Pollen output: With respect to pollen output per anther, a wide variation was observed among the banana genotypes (Fig.1). The variation in pollen output per anther ranged from $3,400 \pm 503.32$ ('Ney Poovan', AB) to $41,400 \pm 1708.80$ ('Athiakol', BB), whereas for the triploids it ranged from $2,200 \pm 683.13$ ('Rasthali', AAB) to $20,200 \pm 886.94$ ('Popoulu', AAB). The mean pollen output per anther was 16,400 and 5,850 in the diploid and triploid cultivars, respectively. Comparatively higher pollen output per anther was observed in the genotypes viz., 'Athiakol' ($41,400 \pm 1708.80$), 'Bhimkol' ($40,200 \pm 600$), 'Popoulu' ($20,200 \pm 886.94$), 'Rose' ($15,600 \pm 516.40$), 'Pisang Lilin' ($15,200 \pm 730.30$), 'Hatidat' ($12,600 \pm 600$), 'Chakkiya' ($11,000 \pm 683.13$) and 'Anaikomban' ($10,600 \pm 200$). Since the quantum of pollen per anther is higher than or similar to that of the proven male parents viz., 'Pisang Lilin' and 'Anaikomban', these genotypes may be grouped as polleniferous types. In earlier studies by Sathiamoorthy (1973 and 1987), superior male fertility level of diploid AA cultivars such as Ambalakadali, Erachivazhai, Tongat and Pisang Lilin was also reported. In the present study, the genotypes viz., 'H-212' ($2,200 \pm 382.97$), 'Rasthali' ($2,200 \pm 683.13$), 'Grand Naine' ($3,200 \pm 461.88$), 'Ney Poovan' ($3,400 \pm 503.32$), 'Bangrier' ($3,600 \pm 230.94$) and 'H-201' ($3,600 \pm 516.40$) recorded lower pollen output per anther. The lower pollen output in case of triploid varieties as compared to that of diploids can be attributed to the imbalance and structural differences of chromosomes as a result of differential genomic constitutions or hybridity and consequential gametic degradation (Adeleke *et al.*, 2004). In case of banana, the diploids are generally known to produce a higher quantum of pollen than the polyploids or the hybrids and are often used in the breeding programs (Sathiyamoorthy, 1994; Dumpe and Ortiz, 1996; Fortescue and Turner, 2004 and Ssebuliba *et al.*, 2008).

Pollen size (μ): Pollen size was recorded in terms of pollen diameter (Fig.2), and was found to have a wide variation among the genotypes. The pollen size in case of diploid cultivars ranged from 87.20μ to 122.74μ and in triploids from 135.24 to 191.11μ . The mean pollen size of the diploid and triploid cultivars was found to be 111.46μ and 167.28μ , respectively. Among the genotypes studied, 'Athiakol' produced pollens of smaller diameter ($87.20 \pm 3.53\mu$), whereas 'Chakkiya' produced pollens of larger diameter ($192.11 \pm 5.35\mu$). Pollen diameter, pollen volume, nucleolar mass and

prophase cell volume were reported to have a direct positive correlation with the ploidy level in many crop species (Srisuwan *et al.*, 2019; Tsukaya, 2013 and Altmann *et al.*, 1994). The observations made on pollen size in the study could be helpful to determine the approximate ploidy status but other sophisticated techniques such as flow cytometry (Dolezel and Bartos, 2005) need to be employed to further confirm the ploidy status.

Pollen viability: The pollen viability (%) as estimated by staining indicated wide variation among the genotypes studied (Fig.3). Overall, the pollen viability ranged from 59.90% to 92.84%, in the diploids whereas in the triploid genotypes, it ranged from 21.62% to 88.76%. The mean pollen viability of the diploid and triploid genotypes was found to be 82.79% and 45.83%, respectively. Higher pollen stainability was recorded by cv. 'Rose' (92.84%), 'Hatidat' (92.23%), 'H-201' (90.52%), 'Chakkiya' (88.76%), 'Sannachenkadali' (86.45%) and 'Pisang Lilin' (85.73%). In the present study, poor viability was recorded in 'Bangrier' (21.62%) and 'H-531' (28.20%). This is in conformity with the earlier reports of Sathiamoorthy (1994) who concluded that the monospecific diploid banana genotypes (*Musa acuminata*) had a better pollen viability than that of bispecific hybrid triploids or tetraploids having genomic constitution of both *M. acuminata* and *M. balbisiana*.

Although stainability provides an estimate of viability, the *in vitro* germinability is considered as the most reliable palynological parameter for estimating the male fertility status. In most of the pollen germination studies, sucrose medium is employed. Since Nyine and Pillay (2007) have reported better results with banana nectar, it was employed in the present study. Wide variation among the genotypes was found in terms of *in vitro* pollen germinability (Fig 3). The pollen germinability ranged from 22.17% ('Ney Poovan') to 85.88% ('Pisang Lilin') in diploids, whereas in the triploids, the germinability was lesser and it ranged from 12.70% ('Grand Naine') to 56.81% ('Chakkiya'). The mean pollen germinability percentages recorded in diploids and triploids were 61.02% and 28.22%, respectively. Higher pollen germination was recorded in polleniferous banana genotypes viz., 'Pisang Lilin' (85.88%), 'Hatidat' (83.38%), 'Rose' (71.09%), 'Anaikomban' (65.59%), 'Athiakol' (61.03%) and 'Bhimkol' (59.71%). In earlier studies, diploid cultivars exhibited pollen germinability percentage up to 60% (Valsalakumari and Nair, 1988). The mean pollen germinability in some of the tetraploid and

diploid genotypes studied by Krishnamoorthy (2002) were 10.38% and 43.78%, respectively.

In the present study, genotypes with poor pollen germination include 'Grand Naine' (12.70%), 'H-531' (14.51%), 'Bangrier' (15.30%), 'Quintal Nendran' (17.24%), 'H-916' (20.43%), 'Nendran' (20.61%) and 'Karpooravali' (21.31%). The bispecific nature of these genotypes could have contributed to meiotic abnormalities such as impaired pairing, abnormal chromosome movement, and unusual sporad configuration leading to poor viability and fertility as earlier described by Sathiamoorthy and Madhav Rao, (1980); Dumpe and Ortiz, 1996), Adeleke *et al.*, (2004), and Ssebuliba *et al.* (2008). Although the germinability is less, frequently normal viable pollens are also encountered in triploids, which can result in successful hybridization. Such was the case in the development of H-201, potential disease tolerant diploid developed in TNAU by involving the triploid cultivar Robusta (AAA) as a male parent (Sathiamoorthy, 1987). In such instances, where the triploids have poor male fertility, they may be employed as female parents to generate secondary tetraploids for further utilisation in breeding programs. Because of gametic restitution often encountered in banana, hybridizing triploid females with diploid males can also result in such tetraploids. Similarly, in the breeding programs in FHIA (Fundación Hondureña de Investigación Agrícola), Honduras several improved banana hybrids such as, FHIA-01 (Prata Ana, AAB × SH-3142, AA), FHIA-02 (Williams, AAA × SH-3393, AA), FHIA-03 (SH-3386, ABB × SH-3320, AA), FHIA-15, 17 & 18 (Highgate hybrids, AAA × Prata Ana hybrids, AAB), FHIA-21 (French plantain hybrids, AAB) and FHIA-23 were developed by Rowe and Rosales (1994).

The association among the observed palynological traits of the banana genotypes revealed that the pollen output per anther is negatively correlated with the pollen size; whereas positively with the pollen stainability and the *in-vitro* pollen germinability (Fig 4). This indicates that the complexities arising out of ploidy status and genomic differences not only impact pollen viability but also pollen production.

Multivariate clustering as represented in the form of heat map (Fig. 5) brought out the persisting variabilities among banana genotypes in terms of the observed palynological attributes. The genotypes taken in the study were grouped into two major clusters based on their palynological traits. Each of the Cluster I and II were further divided



into two sub-clusters. It could be observed that, the genotypes belonging to the major cluster I represented by the genotypes such as 'Rose', 'Pisang Lilin', 'Anaikomban', 'Bhimkol', 'Athiakol' and 'Popoulu', possessed better male fertility as compared to the others. Among these, the diploid cvs 'Rose', 'Pisang Lilin' and 'Anaikomban' have been known as proven male fertile parents and are already being utilized in breeding programmes of TNAU and other centres working on *Musa* improvement. Higher male fertility status as revealed in cvs 'Bhimkol', 'Athiakol', 'Popoulu' and 'Hatidat' is worth exploring for future breeding programmes.

Male fertility in banana is determined by the palynological variants such as pollen output, pollen size, pollen stainability, and pollen germinability. From the present study, it is concluded that the pollen output and pollen viability were higher in monospecific diploids than in the cultivars having the genome of both *Musa acuminata* and *Musa balbisiana*. Pollen size were higher in triploids than diploids. The studies revealed that cvs 'Rose', 'Hatidat', 'Athiakol' and 'Bhimkol' were polleniferous similar to proven male fertile parents such as 'Pisang Lilin' or 'Anaikomban'. Further in-depth studies on the fertility mechanism and identifying possible means to overcome poor fertility status in the triploid banana genotypes would add more value in banana breeding programs.

Acknowledgement

The authors gratefully acknowledge the partly financed 'ICAR-AICRP on Fruits' scheme for the plant materials and the Senior research fellowship extended for the first author for the Ph.D. research program by the Indian Council of Agricultural Research, New Delhi.

References

- Adeleke, M. T., M. Pillay and B. E.Okoli. 2004. Relationships between meiotic irregularities and fertility in diploid and triploid *Musa* L. *Cytologia*, **69**(4): 387-393. <https://doi.org/10.1508/cytologia.69.387>
- Altmann, T., B. Damm, W. B. Frommer, T. Martin, P. C. Morris, D. Schweizer and R. Schmidt. 1994. Easy determination of ploidy level in *Arabidopsis thaliana* plants by means of pollen size measurement. *Plant Cell Reports*, **13**(11): 652-656. <https://doi.org/10.1007/BF00232939>
- Cenci, A., Hueber, Y., Zorrilla-Fontanesi, Y., van Wesemael, J., Kissel, E., Gislard, M., Sardos, J., Swennen, R., Roux, N., Carpentier, S.C. and M. Rouard, 2019. Effect of paleopolyploidy and allopolyploidy on gene expression in banana. *BMC Genomics*, **20**(1): 244. <https://doi.org/10.1186/s12864-019-5618-0>
- Dolezel, J and J. Bartos. 2005. Plant DNA flow cytometry and estimation of nuclear genome size. *Annals of Botany*, **95**(1): 99-110. <https://doi.org/10.1093/aob/mci005>
- Dumpe, B. B and R. Ortiz. 1996 . Apparent male fertility in *Musa* germplasm. *Hort Science*, **31**(6): 1019-1022. <https://doi.org/10.21273/HORTSCI.31.6.1019>
- Fortescue, J and D. Turner. 2004. Pollen fertility in *Musa*: Viability in cultivars grown in Southern Australia. *Australian Journal of Agricultural Research*, **55**(10): 1085-1091. <https://doi.org/10.1071/AR04078>
- Krishnamoorthy, V. 2002. Breeding for resistance to Sigatoka leaf spot and nematodes in banana (*Musa spp.*). Ph.D (Hort.) Thesis submitted to Tamil Nadu Agricultural University, Coimbatore.
- Nyine, M. and M. Pillay. 2007. Banana nectar as a medium for testing pollen viability and germination in *Musa*. *African Journal of Biotechnology*, **6**(10): 1175-1180. <https://doi.org/10.5897/AJB2007.000-2157>
- R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.r-project.org/>
- Rowe, P. and F. Rosales. 1994. *Musa* breeding at FHIA. In: Jones DR (ed) The improvement and testing of *Musa* , a global partnership, Proceedings of the global conference of the international testing programme, FHIA, Honduras, 27-30 Apr 1994, pp 117-129
- Sathiamoorthy, S. 1973. Preliminary investigations on breeding potential of some banana clones. M. Sc.(Ag.) Thesis submitted to Tamil Nadu Agricultural University, Coimbatore.
- Sathiamoorthy, S. 1987. Studies on male breeding potential and certain aspects of breeding bananas. Ph.D (Hort.) Thesis submitted to Tamil Nadu Agricultural University, Coimbatore.
- Sathiamoorthy, S. 1994. The Indian banana breeding program. Paper presented at the Banana and Plantain Breeding: Priorities and Strategies, La Lima (Honduras), 2-3 May. pp. 49-50.
- Sathiamoorthy, S. and V. M. Rao. 1980. Pollen production in relation to genome and ploidy in banana clones. In Muthukrishnan, C. R. and J. B. M. M. A. Khader (Eds.) National seminar on banana production technology: 46-49.



- Tamil Nadu Agricultural University,
Coimbatore.
- Simmonds, N.W. and K. Shepherd. 1955. The taxonomy and origins of the cultivated bananas. Botanical Journal of the Linnean Society, 55 (359): 302 - 312.
- Srisuwan, S., D. Sihachakr, J. Martín, J. Vallès, A. Ressayre, S. C. Brown and S. Siljak- Yakovlev. 2019. Change in nuclear DNA content and pollen size with polyploidisation in the sweet potato (*Ipomoea batatas*, Convolvulaceae) complex. Plant Biology, **21**(2): 237-247.
<https://doi.org/10.1111/plb.12945>
- Ssebuliba, R., A. Tenkouano and M. Pillay. 2008. Male fertility and occurrence of 2n gametes in East African Highland bananas (*Musa spp.*). Euphytica, **164**(1): 53-62.
<https://doi.org/10.1007/s10681-007-9637-6>
- Tsukaya, H. 2013. Does ploidy level directly control cell size? Counter evidence from *Arabidopsis* genetics. PLoS One, **8**(12): 83729.
- Valsalakumari, P.K. and P.C.S. Nair. 1988. Pollen studies in banana. South Indian Horticulture, **36**(3): 96-102.

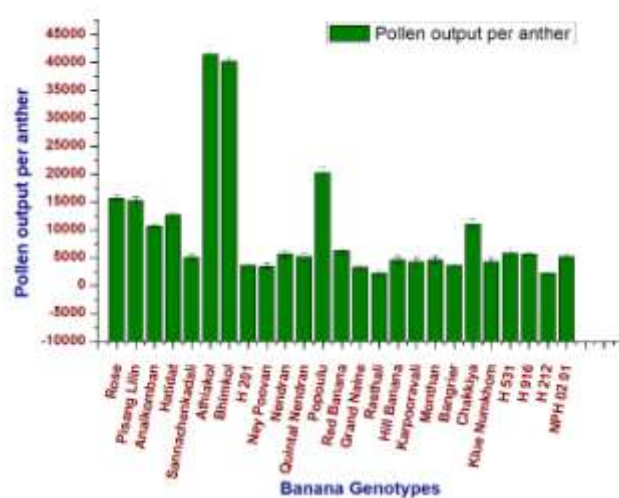


Fig. 1. Pollen output per anther in different banana genotypes

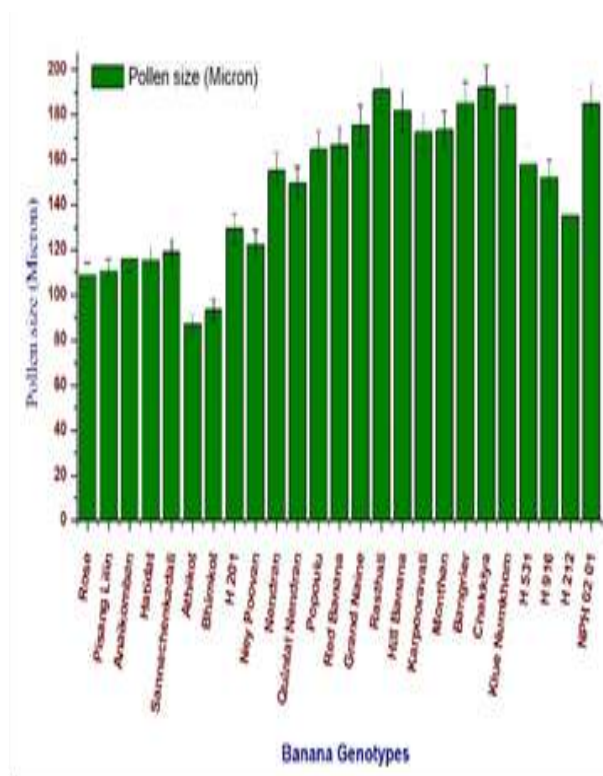


Fig. 2. Pollen size (μ) of different banana genotypes

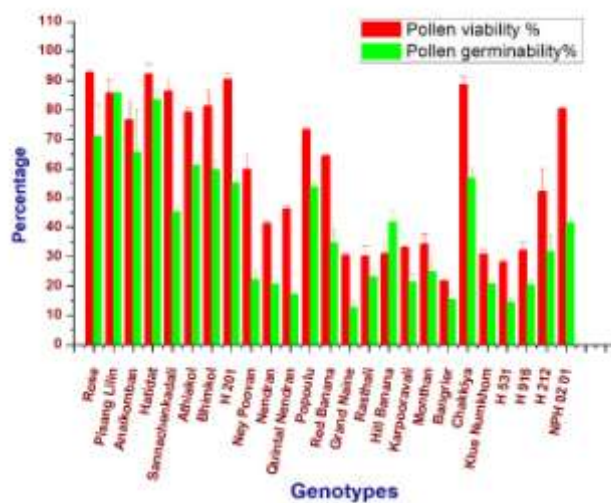


Fig. 3. Pollen stainability % and germinability % of different banana genotypes

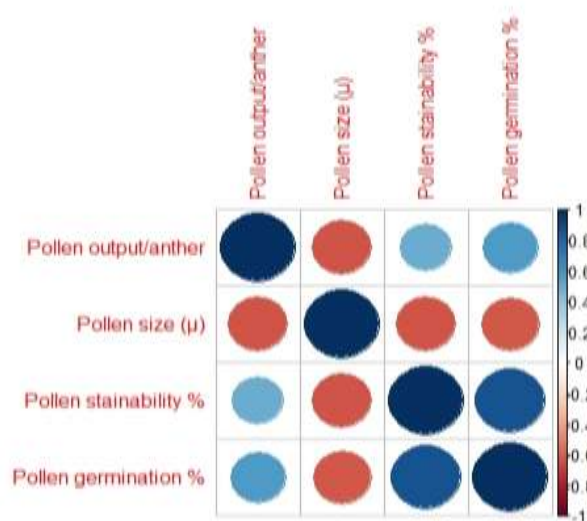


Fig. 4. Graphical representation of the correlation among the palynological traits

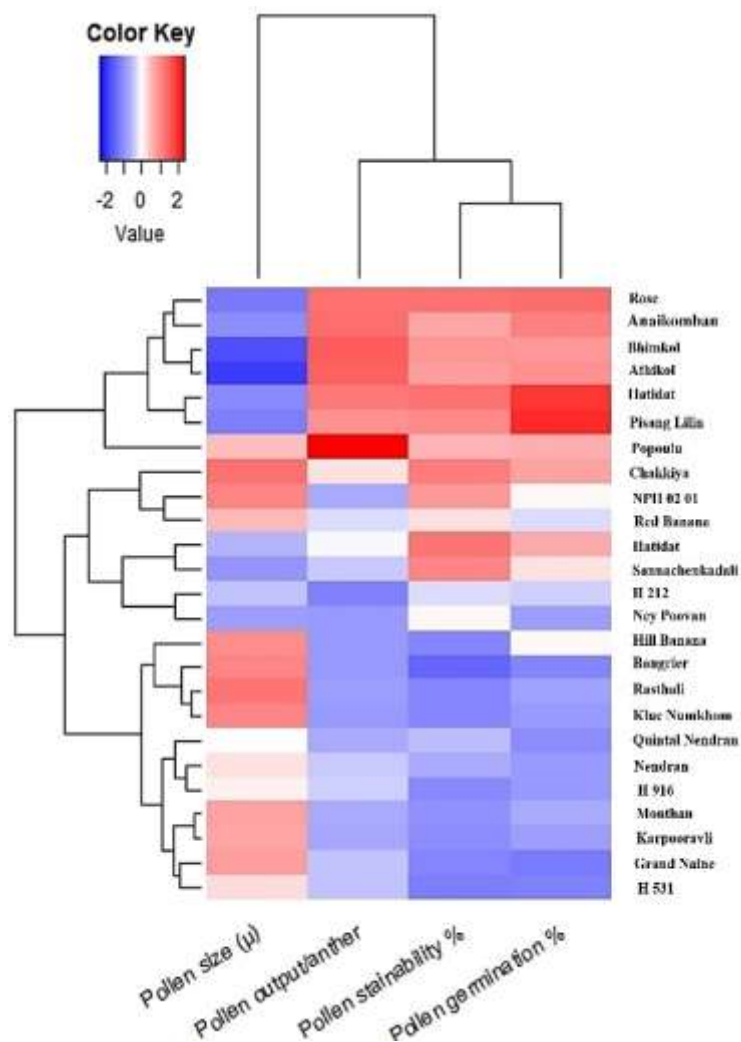


Fig. 5. Heat map depicting clustering of select banana genotypes based on the palynological observations