Author's post-print of the paper published in the Proceedings of the International ISHS-ProMusa Symposium on Unravelling the Banana's Genomic Potential, Acta Horticulturae 1114, ISHS 2016. Available from www.musalit.org

Understanding the Diversity and Reproductive Biology of Banana – for Improvement through Basic Research

S. Uma and K. Arun

National Research Centre for Banana (ICAR), Thogamalai Road, Thayanur Post, Trichy-620102, TamilNadu,India

Keywords: Compatibility, enzymes, fertilization, pollination, seasonal variations and seed set.

Abstract

Success in banana breeding and hybrid development has remained elusive in spite of broad diversity for fertility status. Although key factors, including ploidy, diverse genomic constitution, and male and/or female sterility, have been studied, the basic research to understand their reproductive ecology is limited. Studies on adaptive significance of traits involved in ontogeny in flowering, as well as of factors involved in pollination, fertilization, compatibility, seed production; regeneration, dormancy and so forth need emphasis.Two years' data from NRCB provided a baseline on cross compatibility and improved seed set even under high temperature (by 1-3°C). Two different cultivars were used as female parents ['Kothia' (ABB genome, Bluggoe subgroup) and 'Udhayam' (ABB genome, Pisang Awak subgroup)] and 'Calcutta-4' (*Musa acuminata*) as the male parent. Studies have been conducted to understand the basis of sterility through physical, physiological and biochemical factors affecting stigma compatibility. Time course germination of pollen grains was studied to understand the structural incompatibility regulating seed set. Such basic ecological and morpho-molecular studies, and those on biomolecules involved in reproduction can lead to understanding of mechanisms involved in seed set and could help diversify strategies for banana improvement.

INTRODUCTION

Banana is one of the popular climacteric fruits, valued for its high nutritive value and accessibility to poorer people. The global area under banana cultivation is estimated to be 10.2 million hectares and India is the largest producer with more than 27 million tonnes production (FAOSTAT, 2013). In spite of its increasing area and production, the biotic and abiotic stressespose major problems restricting its spread and productivity.The genetic enhancement of banana against biotic and abiotic stresses through conventional breeding is complicated due to sterility, polyploidy and seedlessness (Simmonds, 1960; De Langhe et al., 2010; Uma et al., 2011). The *Musa* genus possesses low genomic diversity, but not all this diversity can be used for improvement as it is constrained by the above-mentioned weaknesses. In their natural habitats, however, wild *Musa* and related species have contributed to present banana diversity, apart from natural gene introgression. The best strategy of improving vegetatively-propagated crop species such as banana can be combining sexual and asexual reproduction systems to enhance crop diversity with desirable traits.

Generally, plant breeding is based on creating genetic diversity by natural/artificial means and efficient selection of individuals with desirable gene combinations from existing/artificially created populations. Breeding starts with effective pollination accomplished by the transfer of pollen grains from anther to another stigma either within a flower or between the flowers,leading to viable seeds.Banana is a monoecious (contains both male and female reproductive organs) and also dichogamous (temporal and female organs mature before the male organs) (Fortescue and Turner, 2011). This necessitates banana depending on external pollinators like bees and bats under natural conditions. The reproductive accomplishment depends on the number of flowers fertilized and the extent of successful fertilization. Fruit set is a complex process which depends on pollen quantity (Knight et al., 2005), quality, potency in terms of its ability to reach the ovule, compatibility, and female gametophyte potency to develop into a seed and so forth.

These successful mechanisms of pollination can be severely hampered by physical (temperature, humidity and rainfall), physiological (male gametophyte:pollen load size, pollen viability, germination, pattern of anther dehiscence and pollen tube growth; female gametophyte:duration of flowering, flower arrangement, stigma receptivity and effective pollination period) and biochemical factors (enzyme activity, role of plant growth regulators (PGRs) in pollen germination and tube formation,as various genes were involved during the fertilization process) (Ortiz and Vuylsteke,1995; Nepi and Pacini, 2001; Pillay and Tenkouano,2011).

In banana breeding, important factors determining the seed set include its natural ploidy levels (AA, BB, AAB, ABB and ABBB) and their combinations leading to non-parthenocarpic/parthenocarpic progenies (Pillay and Tenkouano, 2011). Basic knowledge regarding physical, physiological and biochemical factors affecting the pollination and fertilization process in banana becomes critical. Hence in this paper, an attempt has been made to synthesise the results of series of experiments carried out on the physical factors influencing the seed set and seed germination,at NRCB, India, under the mega programme on 'Improvement of banana through conventional breeding'.in the context of earlier reports this paper discusses resultsrelating to physiological influence of pollen grains on female gametophyte, efficiency of pollen grains in fresh and stored conditions; identifying the fertile female parents based on pollen germination and seed set; and biochemical influences such as enzymes and proteins in stigma and stylar regions across parents with contrasting fertility status.

MALE AND FEMALE GAMETOPHYTIC DIVERSITY AND COMPLEXITIES

Genetic diversity in banana is restricted to simple morpho-taxonomic traits described in *Musa* logue (IPGRI-INIBAP/CIRAD, 1996), but extends to pollen morphology, fertility status, and detailed morpho-physiological-molecular differences of gametes etc.with reference to breeding. Evaluation of pollen for viability and fertility has indicated significant differences between species, within species, between ploidy levels within a single species, and within ploidy levels of a single species (Sathiamoorthy, 1987; Mukasa and Rubaihayo, 1993; Chin, 1996; Nyine and Pillay, 2007; Krishnamoorthy and Kumar, 2005; Soares et al., 2008). The differences in pollen viability are reported to be as great as 100% between cultivars of the same ploidy level and same species (Fortescue and Turner, 2004).The huge difference in the pollen viability is attributed to the occurrence of irregular meiotic division in

the different ploidy and genomic groups (Sathiamoorthy, 1987).Pooled fertility statuses of various groups and subgroups over a period of seven years, for season, climate and time have been compiled (Fig.1) and a database has been created at NRCB, for genotypic compatibility. Highest fertility of near 100% is recorded with wild *acuminata* and *balbisiana* species and a least or almost nil with Pome and Silk subgroup members.

Although pollen viability is an important factor in identifying a potential male parent, maintenance of vigor for invitro growth and development until reaching the female gametophyte is vital. Pollen viability is only a partial indication while its functional analysis up until complete fertilization reflects true fertility. Rodriguez-Riano and Dafni (2000) reported that not all viable pollen is capable of germinating. Determination of pollen germinationpotential is a good way of selecting improved, disease resistant and pollen fertile parent based on the assumption that pollen capable of germination would be fertile (Barrow, 1983). Thus, studies on pollen kinetics under *in vivo* and *in vitro* conditions were carried out as a part of the basic studies towards understanding the basis of non-setting in banana. Mature excised pollen grains of *Musa acuminata* ssp. *burmannicoides* and *Musa balbisiana* type 'Athiakol' were allowed to germinate in a suitable medium (Soares et al., 2008) and exhibited an average germination rate of 0.05mm/hour but failed to grow beyond 24 hours. Growth culminated with or without the pollen tube bursting. Histological studies of the same pollen grains on seed fertile genotype 'Saba' (ABB genome, Bluggoe subgroup) conducted to understand the kinetics of *in vivo* pollen tube development indicated a growth rate of 3.27mm/hour, several thousand times higher than under invitro conditions (Fig.2). This emphasized that the media used for pollen germination do not mimic the stigmatic surface and our studies indicated that rate of pollen tube growth varies for different female genotypes.This is possible due to the differential physiochemical properties of the stigmatic region influencing pollen germination and pollen tube growth.

STIGMA RECEPTIVITY

Successful pollination in flowering plants depends on complex interactions between the pollen grain and stigmatic surface. Stigma receptivity plays a vital role in the pollination dynamics (Sanzol and Herrero, 2001). The competence of stigma to be receptive is conferred by many internal and external factors about which very little is known with respect to banana. The stigma provides the first entry surface for pollen which facilitates adhesion, hydration and germination. The potential of female gametophytes (sterile/fertile) can be established by rates of pollen hydration, germination and pollen tube elongation in the stigma region. These mechanisms differ based on types of stigma (wet/dry) andthe effective pollination period (Palanivelu et al., 2011). Upon pollen deposition, the stigma plays an important role in pollen germination through secretions containing liquids, gums, sugars and resins. Stigmatic secretion supports pollen protection and avertingstigma desiccation. Successful pollen germination following pollination was evaluated across 218 accessions belonging to AAw, AAcv, BBw, AAA, AAB (Pome, Silk, Plantain, Mysore subgroups and unique genotypes), ABB (Pisang Awak, Peyan, Monthan, Bluggoe subgroups), ABBB cultivars using Calcutta 4 (*Musa acuminata*, non-parthenocarpic / wild) and Pisang Lilin (parthenocarpic - cultivated) as AA pollen parents. Interestingly, seed setting was observed in all cultivars/wilds, except in Cavendish subgroup and very occasionally in Silk subgroup members. This was correlated

with the stickiness of the stigmatic region wheremembers of the Cavendish subgroup were categorized ashaving stigmas with very low stickiness and others as having weakly to strongly sticky stigma groups (Fig.3).

Stigma receptivity relates to a time interval in which pollen is subject to a conducive environment for germination and that encourages pollen competition (Sanzol and Herrero, 2001; Losada and Herrero, 2012).In many crop species it is observed that pollination induced the release of stigmatic fluids as a post-pollination response (Sedgley and Scholefield, 1980; Marginson et.al., 1985) which provided additional nutrients for pollen tube growth whereas Parrie and Lang (1992) evidenced that in blueberry production of stigmatic fluid is stopped when the stigmatic surface was saturated with pollen tetrads. In banana, secretion of stigmatic fluid remains for a period of one to four days depending on the genotype (Fig.4). Minimum of one day was observed in plantain ecotype Nendran (AAB genome, Plantain subgroup) while it extended over a maximum period of four days in cooking bananas (ABB). Thus knowledge on duration of stigma receptivity of the genotypes is of paramount importance for fixing the effective pollination period for the respective genotype to exploit the female parent for maximum seed set. Effect of pollination on seed set on various stages of stigmatic receptivity revealed that in the compatible combinations the female parent has the ability to set the seeds even up to three days after anthesis. But the rate of seed set declined towards later days of anthesis. This indicated that in banana duration of stigmatic receptivity is not a major constraint as pollination can be extended from one to three days and yet resulting in seed set. Studies on nature of stigmatic secretions and their genetic control needs focus for future manipulations in targeted distant crosses in banana.

ENZYMES IN STIGMATIC SURFACE

Despite the nature of stigma surface, in nature, wet and dry stigma types exhibitpresence of enzymes especially high level of esterase and peroxidase activity suggesting their fundamentally important role for stigma function (Zanier and Grilli Caiola, 2000).Preliminary studies were made to estimate the peroxidase and catalase activity in the stigmatic/stylar region of the contrasting genotypes for seed fertility, based on assays (Dafni and Maues, 1998).In general it was observed that even before pollination peroxidase and catalase activity was more in seed fertile cultivars ‘Saba’ (ABB genome, Bluggoe subgroup) and ‘Kothia’(ABB genome, Bluggoe subgroup) than naturally sterile ‘Grand Naine’ (AAA genome, Cavendish subgroup). Higher expression of peroxidase seed fertile genotypes might indicate their highly receptive nature of the stigma than ‘Grand Naine’. Involvement of peroxidase in stigmatic receptivity and its use as an indicator of receptivity has been reported by several workers (Knox, 1984; Galen and Plowright, 1987; McInnis et al., 2006) in *Acacia retinodes*, *Opuntia* and *Seneciosqualidus*. Higher activity of peroxidase enzyme in compatible stigma overself-/incompatible stigma region where peroxidase activity was low or absent has been reported by Carraro et al. (1989). Interestingly new isoforms of peroxidase and catalase were recorded constitutively in stigma of seed fertile cultivars ‘Saba’and ‘Kothia’ and found to be upregulated after pollination while they were absent in the non-seed-setting cultivar ‘Grand Naine’. Differential expression of this peroxidase isoforms might have an important role in stigma receptivity and eventual seed setting efficiency. Earlier report of dual roles of catalases and peroxidases in stress tolerance and calcium signalling,

during pollen germination and tube growth has been reported (Firon et al., 2012). Preliminary studies on role of proteins in stigmatic receptivity and compatibility indicated that fertile stigmas exhibited higher protein contents over sterile stigma. Studies are in progress to study possible role of proteins and the type of proteins.

FACTORS AFFECTING POLLEN GERMINATION

Temperature

In many crop species it is evidenced that temperature is the major climatic factor which influence the pollen germination and tube growth (Hedhly et al., 2005). The negative effects on pollen germination and fruit set due to increasing temperatures during flowering have been reported in several species, including peach (Mellenthin et al., 1980), pear (Sanzol and Herrero, 2001), peanut and sorghum (Prasad et.al., 2003 and 2006). We conducted some invitro studies to know the impact of high temperature on the germination of pollen grains (*M.acuminata* ssp. *burmannicoide*s and *M.balbisiana* type 'Athiakol'). Pollen was pretreated with exposure to higher temperature of 25, 30, 35, 40 and 45°C for 1hour, prior to pollination was imposed. Higher temperatures beyond 35°C reduced the germination percentage from 43-72% but surprisingly, pollen exposed to 45°C still retained their germination efficiency by 28%, if not lost completely. Although reproduction is demonstrated to be the most temperature vulnerable stage in crop plants (Wheeler et al., 2000), our results are indicative that higher temperatures is not a major barrier for pollen germination and banana breeding when all other factors remain conducive. In other words, climate change in terms of higher temperatures may not be a serious constraint for future banana breeding programmes as envisaged in other crops (Wheeler and Braun, 2013).

Pollen Storage

It is well known phenomenon 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) but correct age of the male flower is crucial to improve the seed setting efficiency. Banana is monoecius and lacks synchronization of male and female maturity. In banana breeding, availability of pollen at the right time is crucial. Thus pollen storage is an important complementary breeding activity when the desired genotypes which vary spatially and temporally with respect to flowering. Pollen storage should ensure viability over time which often depends on many factors (Hanna and Towill, 1995; Dafni and Firmage, 2000), of which storage temperature is very important. Long term storage is accomplished with ultra-low temperatures in many crop species (Sedgley and Griffin, 1989) while short term pollen storage is often accomplished at below room temperatures. Studies on pollen storage conditions on viability and germination are limited in banana. Thus experiment was conducted on two ancestral wild species, *M. acuminata* ssp. *burmannicoides* and *M.balbisiana* type 'Athiakol' to know the effect of temperature on pollen viability.Pollen storage was accomplished by storing the whole anthers in eppendorf tubes at three temperature regimes, 0°C, 4°C and room temperature (28-30°C) over a period of 30 days. Among three different storage conditions better result for viability was observed in 4°C (75.2%) then followed by room temperature (52.9%) and 0°C (50.5%)

stored pollen. Among three different stored conditions, 4°C stored pollen grains showed the highest germination percentage (69.4%) followed by room temperature and 0°C (50.6% and 39.4% respectively).Thus pollen stored for four weeks exhibited a cumulative, linear diminution of pollen viability and germination potential over the storage period (Table 1). Although viability was retained upto 30 days, pollen grains failed to germinate beyond 21 days after storage.

Youmbi (2011) working with three *Musa* accessions reported that pollen can be successfully stored up to two months at 10°C. These results clearly indicated that conditions for pollen storage are variety/species specific and banana pollen is not amenable for freeze storage. Crystallization of intracellular water in pollen at low temperatures followed by loss of hydration in the stored pollen grains (Ichikawa and Shiden, 1971) is found to be detrimental for banana pollen grains. But enhanced pollen activity when stored in the ultra-low temperatures like at -20°C in crops like *Betula* sp., *Prunuspersica*, *Medicago sativa* etc. have resulted in supporting germination of pollen even after 9 to 11 years. Thus based on our results and previous studies, it is emphasised that storing at ultra-low temperature should also be exploited for enhancing the storage time and pollen germination efficiency in banana.

FERTILIZATION DYNAMICS

To understand the pollen-stigma interactions, time duration of the pollen tube movement was studied in highly fertile ‘Saba’ and non-seed-setting ‘Grand Naine’ cultivars. In fertilegenotypes pollen germination was observedwithin 5-15 minutes after the pollination and within an hour reached the transmitting tract of the stigmatic region. After 12 hours, the pollen tubes entered the ovary and reached the ovule within 24 hours (Fig.5).Pollen tube passed through the microphylar region and fused with egg cell within 24-48 hours after pollination depending on various factors like dynamics of pollen tube growth like adhesion, nutrition, directional growth, structural hindrances etc. through the ovule.

In seed sterile cv. ‘Grand Naine’, pollen germination was hindered at the stigmatic surface itself while cv. ‘Rasthali’(AAB genome, Silk subgroup) exhibited normal pollen germination on stigmatic surface, pollen tube competition to enter the transmitting tract followed by their organized movement. Sudden disintegration of pollen tubes while traversing through the transmitting tract within 12-24 hours of pollination (Fig.6) could be attributed to possible inhibitorspresent in the conducting trackor absence of some vital elements guiding pollen tube movement. This could be genome dependent. Function of the transmitting tract is to secrete a complex extra cellular matrix composed of several lipids, glycoproteins, and carbohydrates etc. which help directional movement of pollen tube towards the ovule (Cheung et al., 1995; Palanivelu et al., 2003). Among the secreted glycoproteinssome are like s-linked glycoprotein associated with self incompatibility (Franklin-Tong, 1999) is also reported in other crops.

One of the signals generated by pollination and fertilization is the substantial change in the content of several plant hormones, either from the ovary or in later stages, of the embryo. But till date the mechanisms underlying growth control by these hormones in pollination dynamics remains unclear. To understand the possible influence of hormones with respect to pollen germination and pollen tube growth, different hormones like zeatin, abscisic acid (ABA), IAA and GA_3 were estimated in the stigmatic region of contrasting genotypes

(seed sterile and fertile). The results revealed that, in general, the level of all the hormones was more in fertile genotype compared to non-seed-setting genotype and interestingly very low level of hormone concentrations was observed in 'Grand Naine' where the pollination was prevented at pollen germination itself.But, the maximum level of GA_3 concentration was recorded in the other non-seed-setting genotype 'Rasthali', where at least pollen tube growth was observed on the stigmatic region in initial stage of the pollination. Singh et al. (2002) proved GA_3 are required for normal in pollen tube growth while Wu et al. (2008) reported that IAA was the most effective in stimulating pollen tube growth and causing the shank part of pollen tubes to be slender and straight. In our study also, except for 'Grand Naine', IAA was recorded in all other genotypes where the pollen tube growth was observed (Fig.7).Result with 'Rasthali' suggested that GA_3 hormone could be necessary for pollen tube growth.

In general, maximum seed set was observed in the apical region of the ovary, irrespective of the cultivars and ploidy levels of the female parent which revealed the important role of pollen tube growth in influencing the seed setting. Shepherd (1960) also stated that long distance travel is a problem for pollen tubes and often leading to a hindrance to seed set. Thus it is believed that shorter style cultivar have a more seed setting ability when compared to long style cultivars. But present study disproved this hypothesis as the high-seed-setting cultivar'Saba' has longer style length (4.6-4.7cm) when compared to the non-seed-setting cultivar 'Grand Naine'(4.1-4.3cm). This suggested that physical parameter like style length may not have a major role while other factors like stigma receptivity and ovule receptivity might also be playing a major role in seed setting.

To understand the effect of environmental factors with respect to seed set and germination, efforts were taken to analyze the ten year data on seed setting in two different locations, Kerala and Tamil Nadu which enjoy tropical humid weather and dry weather respectively. Across the edible banana groups, subgroups and genotypes, the highest seed set has been reported in cooking types (ABB) in humid Kerala climate while Pisang Awaks recorded maximum seed set in dry weather conditions of Tamil Nadu. Similarly there was a differential seed setting nature across the locations was recorded. This suggested that the success of the seed set not only genotype dependent but environmental factors exert a greater influence the seed setting potential of the genotypes (Table 2). Thus it is emphasized earlier reports (Swennen and Vuylsteke, 1990; Swennen et al., 1991, 1992; Ssebuliba et al., 2009) that the performance of the cultivars will vary depends on the environmental factors. The pattern of monthly variation for seed set in cultivars 'Kothia'and 'Udhayam' (ABB genome, Pisang Awak subgroup) resulted in maximum seed set during the month of July characterized by high rainfall, high humidity and low moderate temperatures (24-26°C) at Kerala (Agali) while at Trichy, during the month of April characterized by high temperature, low humidity and scanty rainfall. The variations can be attributed to differences in weather patterns suggesting that temperature and relative humidity influence seed set. Ortiz and Vuylsteke, (1995) also reported that, in general, better seed set plantain cultivars is observed with under high relative humidity and low temperatures whereas higher recovery of tetraploids was favored by low relative humidity, higher temperature and solar radiation. Though the seed set was high during the month of April, (peak summer in Tamil Nadu), the plants derived showed abnormalities in their structure and growth suggesting their euploidy status. This is

supported by earlier works of Ortiz et al. (1998) indicating that high temperature, high solar radiation with low humidity condition will lead to unbalanced gamete production and Adeleke et al., (2004) have reported the meiotic irregularities including lagging chromosomes and univalent formation influencing the fertility in *Musa*. Similarly, Shepherd et al. (1994) also proved that the pollinations occurring during the dry summer produce hybrids with undesired ploidies, such as hexaploids and heptaploids, while pollinations in cooler and more humid seasons have lower seed yield but a higher tetraploid recovery rate. These results reveal that breeder should consider the choice of the month/season based on geographic locations, apart from choice of the parents which have more impact on the seed set and guarantee the efficiency in *Musa* cross-breeding.

CONCLUSIONS

Success of banana breeding and hybrid development has remained elusive in spite of broad crop diversity. Banana breeding is very complex for various plant and system based complexities. Basic studies on ecological, morpho-molecular and bio-molecules involved in reproduction can lead to better understanding of the mechanism/s involved in seed set. Our studies and previous reports and results from other crop plants indicate that pollination manipulations through physical, hormonal and biochemical means could aid in seed set in sterile genotypes and better seed set fertile genotypes. Recent results at NRCB have also indicated improved seed set by 33% through external chemical sprays. It is well demonstrated that development of superior hybrids in banana is possible with improved process of breeding aided by genomic tools for a better product delivery.

ACKNOWLEDGEMENTS

Authors thank ICAR, New Delhi and Director, NRCB for providing support to carry out this work and having permitted to present during the conference. We also thank Drs. S. Backiyarani, M.S. Saraswathi, P.Durai for their help and valuable suggestions during the work. Ms. Sivasankari, M.Kumaravel, I. Prabhavathi, A.Mariadoss, K.P. Sajith and S. Lakshmi for their support. Author K.Arun is a PhD scholar at NRCB supported by Bioversity International, France.

Literature Cited

Anonymous, 2013.Annual Report, National Research Centre for Banana (ICAR), Tiruchirapalli, Tamil Nadu, India.

Adeleke, M.T.V., Pillay, M. and Okoli, B.E. 2004. Relationships between meiotic irregularities and fertility in diploid and triploid *Musa* L. Cytologia 69:387–393.

Barrow, J.R.1983. Comparisons among pollen viability measurements in cotton. Crop Sci. 23:734-736.

Carraro, L., Gerola, P.D., Lombardo, G. and Gerola, F.M. 1989. Peroxidase activity and gametophytic incompatibility: bud-pollination in *Petunia Hybrida*. Caryologia 42 (3-4):225-234.

Cheung, A.Y., Wang, H. and Wu, H.M. 1995. A floral transmitting tissue-specific glycoprotein attracts pollen tubes and stimulates their growth. Cell 82: 383–393.

Chin, H.F.1996. Germination and storage of banana seed.p. 218-227. In: E.A. Frison, J.P. Horry and D. De Waele (eds). New Frontiers in Resistance Breeding for Nematode, Fusarium and Sigatoka. INIBAP, Montpellier.

Dafni, A and Maues, M.M. 1998.A rapid and simple procedure to determine stigma receptivity. Sex. Plant Reprod. 11:177–180.

Dafni, A. and Firmage, D. 2000. Pollen viability and longevity: Practical, ecological and evolutionary implications. Plant Syst.Evol. 222:113-132.

De Langhe, E., Hribova, E., Carpentier, S., Dolezel, J. and Swennen, R. 2010. Did backcrossing contribute to the origin of hybrid edible bananas?. Annals of Botany 106: 849–857.

IPGRI-INIBAP/CIRAD.1996.Descriptors for Banana (*Musa* spp.). IPGRI, Rome/ INIBAP, Montpellier/CIRAD, Montpellier.

FAOSTAT, 2013.Food and Agricultural Commodities Production.Food and Agricultural Organization Statistics. http://faostat3.fao.org/home/E.

Firon, N., Nepi, M. and Pacini, E. 2012. Water status and associated processes mark critical stages in pollen development and functioning. Annals of Botany109:1201–1213.

Fortescue, J.A. and Turner D.W. 2004.Pollen fertility in *Musa*.Viability in cultivars grown in Southern Australia.Aust.J. Agr. Res.55:1085-1091.

Fortescue, J.A. and Turner D.W. 2011.Reproductive biology. p. 145-180. In: M. Pillay and A. Tenkouano (eds), Banana Breeding - Progress and Challenges, Chap. 8. CRC Press, London.

Franklin- Tong, V.E. 1999. Signaling and the modulation of pollen tube growth. The Plant Cell 11(4):727-738.

Galen, C. and Plowright, R.C. 1987. Testing the accuracy of using peroxidase activity to indicate stigma receptivity.Rev. Can. Bot. 65(1):107-111.

Hanna, W.W. and Towill, L.E. 1995. Long-term pollen storage. Pp. 179–207 in J. Janick (ed.). Plant Breeding Reviews Vol.13. John Wiley & Sons, London.

Hedhly, A., Hormaza, J.I.and Herrero, M. 2005. The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. Plant Biol.(5):476-83.

Ichikawa, S. and Shiden, T. 1971.Fundamental studies on deep freezing storage of the tree pollen.Kyoto Univ. Bull. 42:51-82.

Knight, T. M., Steets, J.A., Vamosi, J.C., Mazer, S.J., and Bure, M. 2005. Pollen limitation of plant reproduction: pattern and process.Annu Rev. Ecol. Evol. S. 36:467-497.

Knox, R.B. 1984. Pollen–Pistil interactions. p.508-608. In: H.F. Linskens, J. Heslop-Harrison (eds), Cellular Interactions (Encyclopedia of Plant Physiology, Vol. 17), Springer, New York/Berlin/Heidelberg.

Krishnamoorthy, V. and Kumar, N. 2005. Breeding potential of new synthetic banana hybrids. PGR Newsletter 144:63-66.

Losada J.M. and Herrero M. 2012.Arabinogalactan-protein secretion is associated with the acquisition of stigmatic receptivity in the apple flower. Ann. Bot. 110:573-584.

Marginson, R., Sedgley, M. and Knox, B.B. 1985.Physiology of post-pollination exudates production in *Acacia.* J. Exp. Bot. 36:1660-1668.

McInnis, S.M., Desikan, R., Hancock, J.T. and Hiscock, S.J. 2006. Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: potential signalling crosstalk?.New Phytol. 172:221–228.

Mellenthin, W.M., Chen, M.P. and Kelly, B.S. 1980. Blow oxygen effects on desert quality, scald prevention and nitrogen metabolism of ‘d’Anjou’ pear fruit during long-term storage. J. Am. Soc. Hort. Sci. 105:695-698.

Mukasa, B.S. and Rubaihayo, R.P. 1993. Male fertility in Uganda banana germplasm. Afr. Crop Sci. J. 1(2):87-93.

Nepi, M. and Pacini, E. 2001. Effect of pistil age on pollen tube growth, fruit development and seed set in *Cucurbita pepo* L. Acta.Soc.Bot.poloniae.3:165-172.

Nyine, M. and M.Pillay. 2007. Banana nectar as a medium for testing pollen viability and germination in *Musa*. Afr. J. Biotechnol.6:1175-1180.

Ortiz, R. and Vuylsteke, D. 1995. Factors influencing seed set in triploid *Musa* ssp.L. and production of euploid hybrids. Ann. Bot. London. 75:151–155.

Ortiz,R., Ulburghs.F. and Okoro. U. J 1998. Seasonal variation of apparent male fertility and 2n pollen production in plantain and banana. Hortscience 33(1):146-148.

Palanivelu, R., Brass, L., Edlund, A.F. and Preuss, D. 2003. Pollen tube growth and guidance is regulated byPOP2, an *Arabidopsis* gene that controls GABA levels. Cell. 114:47–59.

Palanivelu, R. and Tsukamoto. 2011. Path finding in angiosperm reproduction: pollen tube guidance by pistils ensures successful double fertilization. WIREs Dev Biol. 1:96-113.

Parrie, E.J., and G.A. Lang. 1992. Self- and cross-pollination affect stigmatic pollen saturation in blueberry. Hort Science. 27:1105-1107.

Pillay, M. and Tenkouano, A. (eds). 2011. Banana Breeding - Progress and Challenges.CRC Press, London.

Prasad, P.V.V., Boote, K.J., Allen Jr., L.H. and Thomas, J.M.G.2003.Super-optimal temperatures are detrimental to peanut (*Arachishypogaea* L.) reproductive processes and yield at both ambient and elevated carbon dioxide.Glob.Change Biol. 9:1775-1787.

Prasad, P.V.V., Boote, K.J. and Allen, L.H. 2006. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [*Sorghum bicolor* (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. Agr.forestmeteorol. 139(3):237-251.

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

Sanzol, J. and Herrero, M. 2001. The "effective pollination period" in fruit trees. Sci. Hort. 90:1-17.

Sathiamoorthy, S. 1987. Studies on Male Breeding Potential and Certain Aspects of Breeding Bananas.PhD dissertation.Tamil Nadu Agricultural University, Coimbatore.

Sedgley, M. and Griffin, A.R. 1989. Sexual Reproduction of Tree Crops.Academic Press, Kent.

Sedgley, M. and Scholefield, P.B. 1980.Stigma secretion in the watermelon before and after pollination. Bot. Gaz. (Chicago) 141(4):428-434.

Shepherd, K, Dantas, J.L.L. and de Oliveira e Silva, S. 1994.Breeding Prataand Maçã for Brazil.p. 157-168. In: D.R. Jones (ed.), The Improvement and Testing of *Musa*: a Global Partnership. INIBAP, Montpellier.

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

Shivanna, K.R. and Johri, B.M. 1989. The Angiosperm Pollen: Structure and Function. John Wiley and Sons.

Simmonds, N.W. 1960. Megasporogenesis and female fertility in three edible triploid bananas. J. Genet. 57:269-278.

Singh, P.D., Jermakow, M.A. and Swain, M.S. 2002. Gibberellins are required for seed development and pollen tube growth in *Arabidopsis*. Plant Cell 14(12):3133-3147.

Soares, T.L.,Silva,S.O., Costa,M.A.P.C., Serejo,J.A.S., Souza, A.S., Lino, L.S.M., Souza, E.H. and Jesus,O.N. 2008.*In vitro* germination and viability of pollen grains of banana diploids.Crop Breed..Appl. Biot.8:111-118.

Ssebuliba, R.N., Magambo, M. and Pillay, M. 2009. Patterns of seed set in East African Highland banana (*Musa* sp.) hybrids. J. New Seeds 10:160-170.

Swennen. R. and Vuylsteke, D. 1990. Aspects of plantain breeding at IITA.p. 252-266. In: R.A. Fullerton and R.H. Stover (eds), Sigatoka Leaf Spot Diseases of Bananas. INIBAP. Montpellier.

Swennen. R., Vuylsteke, D. and De Smet, K. 1991. Season dependent seed set in plantain. Banana Newsletter 14:35-36.

Swennen. R., Vuylsteke, D. and Hahn, S.K. 1992. The use of simple biotechnological tools to facilitate plantain breeding.p. 69-74. In: G. Thottappilly, L.M. Monti, D.R. Mohan Raj, and A.W.Moore (eds), Biotechnology: Enhancing Research on Tropical Crops in Africa. CTA, Wageningen, IITA, Ibadan.

Uma, S., Mustaffa, M.M., Saraswathi, M.S. and Durai, P. 2011. Exploitation of diploids in Indian banana breeding programmes. Acta Hort. 897:215-223.

Wheeler, T.R.,Craufurd, P.Q., Ellis, R.H., Porter, J.R. and Vara Prasad, P.V. 2000. Temperature variability and the yield of annual crops. Agric. Ecosyst. Environ. 82:159–167.

Wheeler, T. and von Braun, J. 2013. Climate change impacts on global food security.Science 341(6145):508-513.

Wu, J.Z., Lin, Y., Zhang, L.X., Pang, W.D.and Zhao. J.2008. IAA stimulates pollen tube growth and mediates the modification of its wall composition and structure in *Toreniafournieri.* J Exp. Bot. 59(9):2529-2543.

Youmbi, E, Fonkam, N.J.P., Tomekpe, K. and Fonbah. C. 2011. *In vitro* germination and pollen conservation of some *Musa* species.Asian J. Biotech. 3:554-563.

Zanier, R. and M. Grilli Caiola.2000.Self incompatibility mechanisms in the *Crocus sativus* aggregate *(Iridaceae)*: a preliminary investigation. Annali di Botanica.55:84-90.

Tables

Table 1.Effect of pollen storage on pollen viability and germination.

Stored Pollen Grains (Days)	Viability						Germination					
	0°C		4°C		Room Temperature		0°C		4°C		Room Temperature	
	AA	BB	AA	BB	AA	BB	AA	BB	AA	BB	AA	BB
7 days	60.8[b]	40.2[b]	80.2[b]	70.1[b]	55.5[b]	50.2[b]	40.2[b]	38.5[b]	70.2[b]	68.5[b]	50.6[b]	50.6[b]
14 days	50.2[c]	38.2[c]	70.2[c]	60.5[c]	40.1[c]	38.2[c]	20.6[c]	18.6[c]	30.2[c]	28.9[c]	19.5[c]	24.6[c]
21 days	40.8[d]	35.8[d]	65.2[d]	58.4[d]	38.1[d]	32.6[d]	11.2[d]	10.6[d]	19.8[d]	18.6[d]	10.4[d]	11.8[d]
30 days	36.2[e]	30.5[e]	58.7[e]	52.6[e]	30.5[e]	28.5[e]	0	0	0	0	0	0
Fresh	90.6[a]	80.6$_a$	90.6[a]	80.6[a]	90.6[a]	80.6[a]	92.4[a]	80.4[a]	92.4[a]	80.4[a]	92.4[a]	80.4[a]

Means having the same letters in column do not different significantly at $P<0.05$ (Duncan's Multiple Range Test).

Table 2. Female fertility based on the pollen germination on stigma region and seed set.

Name	Genome-Subgroup	Female fertility based on the pollen germination on stigma region and seed set
Chendgwat, PisangJajee	AAw	++++
SCK, Anaikomban	AAcv	+++
Attikol, Elavazahi	BBw	++++
Manoranjitham	AAA	+
Rasthali and Kozhikodu	AAB-Silk	+
Marabale	AAB-Pome	++
Alphon	AAB-Mysore	++
Karpuravalli, Bankela	ABB-PisangAwak	+++
Saba,Kothia	ABB-Bluggoe	+++
Monthan	ABB-Monthan	++
Bhat Monahar, Foconah	ABBBcv	+++

Figures

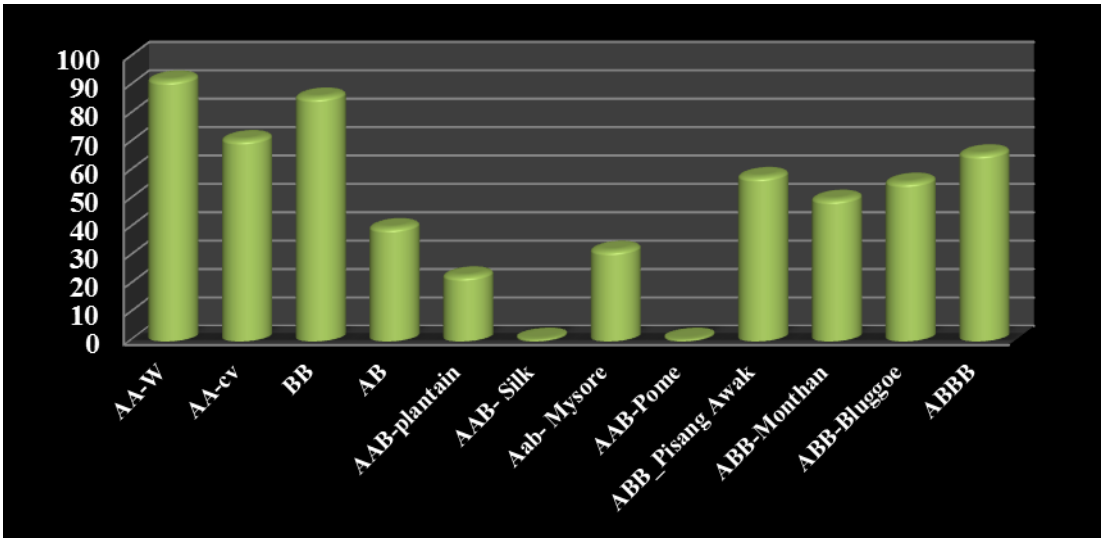


Fig. 1. Percent pollen fertility across *Musa* groups and subgroups.

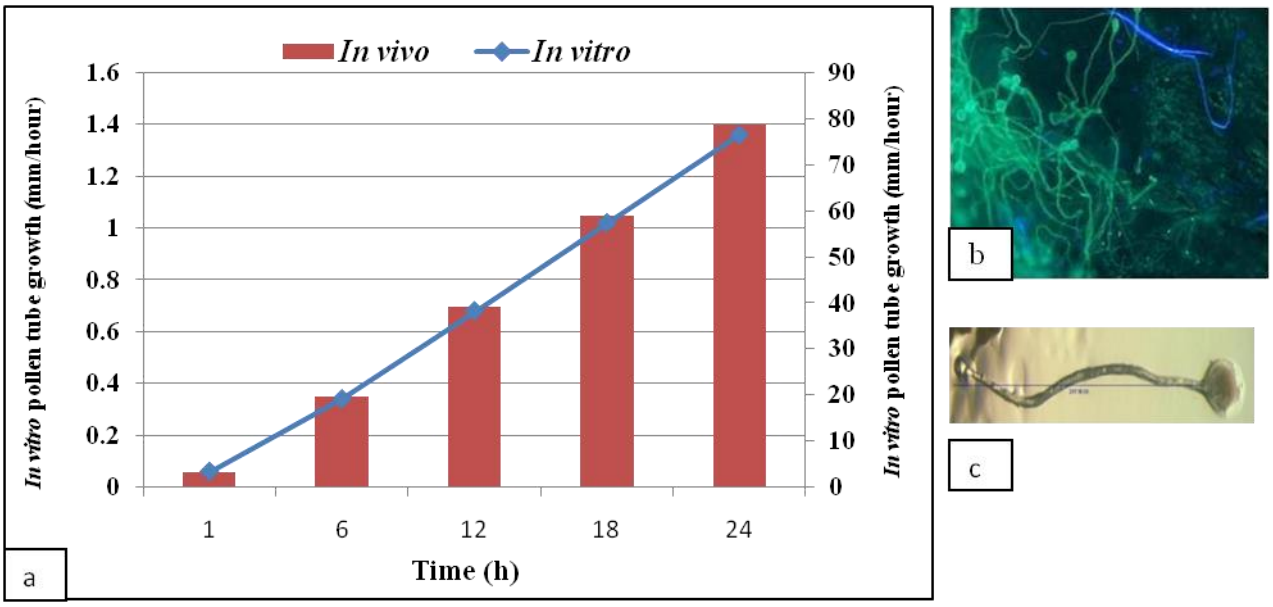


Fig. 2. a) Kinetics of in vivo and in vitro pollen tube growth in different time intervals (across groups and subgroups). b) In vivo pollen germination in transmitting tube. c) In vitro pollen germination.

Fig. 3. a) Non-sticky and b) sticky stigmatic surfaces.

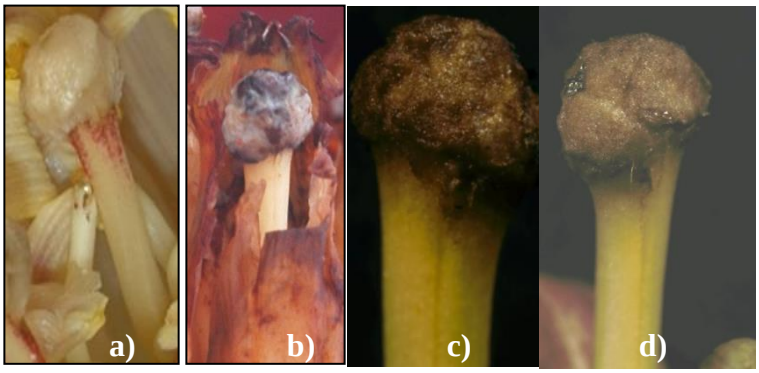


Fig. 4. Stigma at various stages of receptivity in banana Calcutta 4 (AA).

Stigma region - Pollen germinates 5-15 m after pollination

Transmitting tract- 1 hour after pollen tubes enter into the stylar region and passes through the

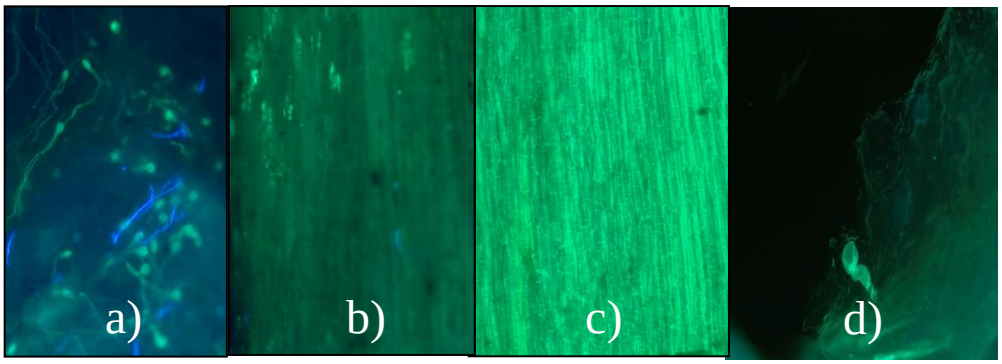
Ovary- After 12 hours pollen tubes enter into the ovary

Between 12-24 hours pollen tube goes round the ovule

Between 24-48 hours - Fertilization- Pollen tube passes through the microphylar region and fuses with egg cell

So, any stress between 0-48 hours is crucial for pollination, fertilization and seed set in banana

Fig. 5. Approximate time duration of movement of pollen (tube) into the ovule across crosses.



a) 'Rasthali' - Pollen germination in pollen tube
b) 'Rasthali' - Pollen tube growth in style
c) 'Rasthali' - Disintegrating pollen tubes after 24 hours
d) 'Grand Naine' - Pollen germination barrier at stigmatic surface

Fig. 6. Pollen tube growth pattern in non-seed-setting banana genotypes, Rasthali (AAB genome, Silk subgroup) and Grand Naine (AAA genome, Cavendish subgroup).

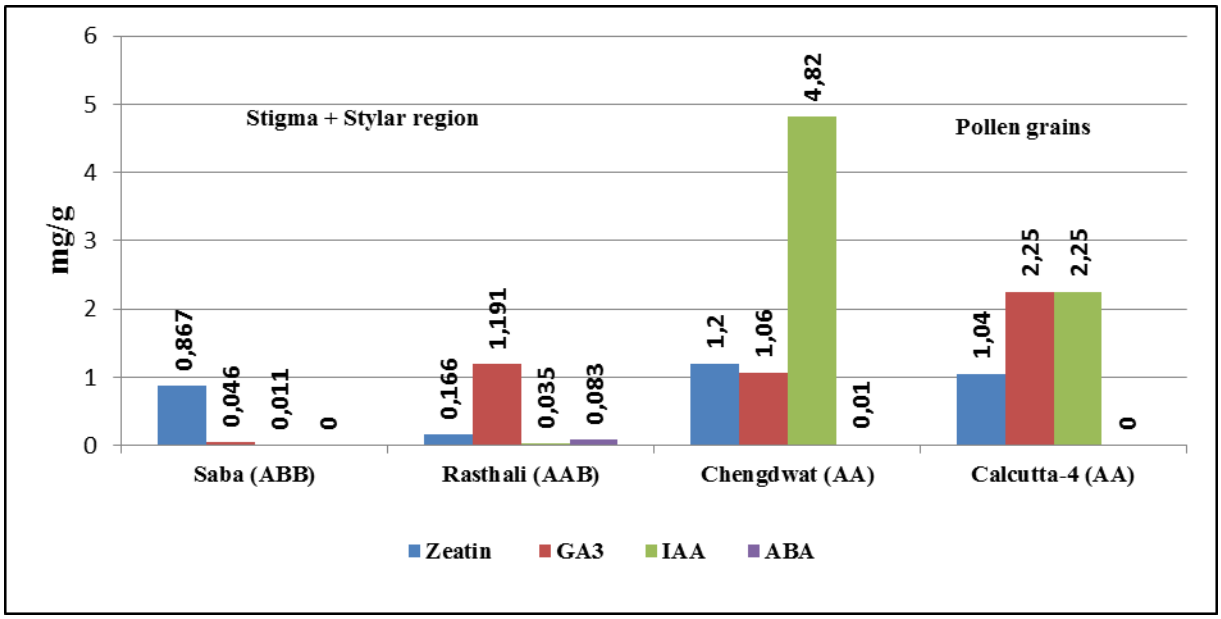


Fig. 7. Level of plant growth regulators in pollen grains and pistil.