The mission of the **International Network for the Improvement of Banana and Plantain** is to increase the productivity and stability of banana and plantain grown on smallholdings for domestic consumption and for local and export markets.

INIBAP has four specific objectives:

- to organize and coordinate a global research effort on banana and plantain, aimed at the development, evaluation and dissemination of improved cultivars and at the conservation and use of *Musa* diversity;
- to promote and strengthen regional efforts to address region-specific problems and to assist national programmes within the regions to contribute towards, and benefit from, the global research effort;
- to strengthen the ability of NARS to conduct research on bananas and plantains;
- to coordinate, facilitate and support the production, collection and exchange of information and documentation related to banana and plantain.

In May 1994, INIBAP was brought under the governance and administration of the International Plant Genetic Resources Institute (IPGRI) to enhance opportunities for serving the interest of small-scale banana and plantain producers.

The International Plant Genetic Resources Institute is an autonomous international scientific organization operating under the aegis of the Consultative Group on International Agricultural Research (CGIAR). The international status of IPGRI is conferred under an Establishment Agreement which, by December 1995, had been signed by the Governments of Australia, Belgium, Benin, Bolivia, Burkina Faso, Cameroon, China, Chile, Congo, Costa Rica, Côte d'Ivoire, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Greece, Guinea, Hungary, India, Iran, Israel, Italy, Jordan, Kenya, Mauritania, Morocco, Pakistan, Panama, Peru, Poland, Portugal, Romania, Russia, Senegal, Slovak Republic, Sudan, Switzerland, Syria, Tunisia, Turkey, Ukraine and Uganda. IPGRI's mandate is to advance the conservation and use of plant genetic resources for the benefit of present and future generations. IPGRI works in partnership with other organizations, undertaking research, training and the provision of scientific and technical advice and information, and has a particularly strong programme link with the Food and Agriculture Organization of the United Nations. Financial support for the agreed research agenda of IPGRI is provided by the Governments of Australia, Austria, Belgium, Canada, China, Denmark, France, Germany, India, Italy, Japan, the Republic of Korea, Mexico, the Netherlands, Norway, Spain, Sweden, Switzerland, the UK and the USA, and by the Asian Development Bank, IDRC, UNDP and the World Bank.

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New Frontiers in Resistance Breeding for Nematode, Fusarium and Sigatoka

Proceedings of the workshop held in Kuala Lumpur, Malaysia 2-5 October 1995

Edited by
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and D. De Waele

IPGRI CIRAD MARDI inibap

Germination and Storage of Banana Seeds

H.F. Chin

Introduction

Most people are surprised to learn that banana is the fourth most important global food commodity after rice, wheat and milk in terms of gross value of production (Tribe 1994). In spite of its importance, *Musa* research is the Cinderella of the global agricultural research network. Many problems require investigation. *Musa* improvement through breeding is difficult, as hybridization is rarely possible and the testing of a new variety takes 2 or 3 years. The transfer of germplasm is associated with high risks, and quarantine procedures take months. Considering all these difficulties, it is understandable that banana and plantain are the least-researched major food commodities.

Numerous developments in the past decade have changed the scenario for genetic conservation of *Musa*, particularly the establishment of INIBAP. Many varieties of banana and plantain exist all over the world, and it is believed that many problems such as disease and pest susceptibility can be solved by the selection of existing resistant varieties and the breeding of new varieties. Improvement of *Musa* has, so far, been relying on a very narrow genetic base and broad germplasm collections are required.

For a seed-propagated crop, a base collection in a genebank will consist of seeds dried and stored at subzero temperatures. An active collection consists of seeds stored at above 0°C. In the case of *Musa* genetic conservation, different types of genebanks (*in vitro*, field collection) are utilized which complement each other. There is a need to further complement the clonal collections by seed storage. In this paper, the morphology and structure of various banana seeds are described. Germination of *Musa* seeds, their viability and effects of moisture and temperature were studied. Results from this study can be of use during collecting missions and can be applied to work in genebanks and by plant breeders.

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Collection and distribution of *Musa* species

Three species of wild banana—*Musa acuminata*, *M. gracilis* and *M. violascens*—were recognized in Peninsular Malaysia by Simmonds (1955) (Table 1). Argent (1976) recorded nine species in Papua New Guinea (Table 2). He considered banana very difficult to collect as herbarium specimens and the species will remain poorly represented. The main field collections of banana germplasm are in Honduras, Jamaica, Philippines, Papua New Guinea and India (IBPGR 1978). Many small collections maintain limited materials in Africa, the Americas and Asia[1]. Siti Hawa Jamaluddin (1986) reported that there are about 138 banana cultivars in the germplasm collection in Malaysia (MARDI). A collection of germplasm is necessary to provide a wide genetic base as support for a breeding programme. An international *Musa* germplasm exchange system has been established through which varieties and wild species are made available. It is important to have an information system internationally useful and common to all breeders. Also, duplicates of accessions stored in a different country are necessary as a further protection and security to conservation.

Guidelines for seed collecting are useful for those involved in collecting for a seedbank. Furthermore, ripe seed samples are important, as are field observations for the taxonomic identification of *Musa* species.

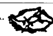
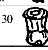
The number of seeds in a fruit is fairly large, from 30-130, and thousands of seeds can be obtained from a bunch. It is important to know that seeds can be collected even from an immature fruit but seeds have to be black and hard, even if the fruit is not ripe but turning yellow. It is rare to see ripe fruits on a bunch in the wild state; usually they are eaten by animals. It is best to extract seeds after ripening of collected fruits. After extraction, they have to be washed and cleaned; many remain moist for 1-2 weeks but they have to be dried if they are to be stored for a couple of months, or transported at high altitude and low temperature over long distances. After drying to 8-10% moisture content, seeds can be packed in sealed containers and stored at 5°C for medium-term storage or at -18°C for long-term storage.

Morphology and anatomy of seed

Many of the wild species and some varieties of banana produce seeds, and are thus propagated from seeds. In the wild state, they are eaten and dispersed by animals; those that are buried in the soil can remain viable for long periods. It is important to be able to identify seeds of different species and cultivars for breeding purposes and taxonomical classification in seedbanks. The seed and embryo of the genus *Musa* were studied and described by Humphrey (1986).

[1] Editor's note: a recent survey organized by INIBAP in 1995 showed a significant change in the relative importance of *Musa* collections. See table provided in Annex 1 of this paper.

Table 1. Fruits and seeds of *Musa* species and varieties

Musa species/ variety	Seed origin	Seed moisture (%)	1000-seed weight (g)	Seed description	Seed size (mm)	Fruits	Seeds/ fruit	Seed shape
M. acuminata var. Kra	Malaysia	40	68	Black, triangular or 4 sided	6x5	35 fruits/bunch; wt/bunch 600g; wt/fruit 17g	48	
M. Ensete glauca	Thailand/ Indonesia	15	627	Brownish black, roundish, flat side	22x11	N.A.	N.A.	
Var. Penk	Malaysia	29	38	Light brown, roundish, flat top and bottom	4-5 diam	70 fruits/bunch; wt/bunch 5718g	31	
M. violascens	Malaysia (Sungai Buloh)	31	92	Brownish/black, long cylindrical	4X4.5	60 fruits/ bunch	70	
M. acuminata wild	Malaysia	10	46	Brownish black, roundish flat	5-6 diam.	231 fruits/bunch; wt/bunch 4990g	67	
Var. Gala	Malaysia (Kelantan)	11	39	Light brown, roundish	6 diam.	52 fruits/bunch	50	
M. ornata	Indonesia	15	29	Light grey, irregular, 4 sided	4-5	N.A.	N.A.	
M. velutina	Indonesia	15	41	Light brown, irregular 4 sided	6x3	N.A.	N.A.	
M. gracilis	Malaysia (Endau Rompin)	46	94	Brownish black, rounded cylinder	7X4	16 fruits/bunch	28-130	

NA: Not available

Table 2. A collection of banana seeds in herbarium and cold storage

Banana species	Collection	No.
M. acuminata ssp. *banksii*	PNG[1]	060*, 174*, 180*
	PNG	291, 292
	PNG	343, 344, 363
	PNG	220
M. acuminata ssp. *banksii*	Australia	
ssp. red variant	Lawang, East Java	
M. acuminata (wild)	Malaysia	
var. *Kra*	Malaysia (Genting)	
var. *Gala*	Malaysia (Ulu Kelantan)	
M. boman	PNG	341
M. glauca	Thailand	
M. ingens	PNG	200
M. jackeyi	Australia	
M. lolodensis	PNG	364
M. maclayi	PNG	314, 339*
M. ornata	Indonesia	
M. peekelii	PNG	315, 316
subsp. *angustigemma*	PNG	150
M. schizocarpa	PNG	320, 329, 232*, 6042*
M. velutina	Malaysia/Indonesia	

[1] PNG Papua New Guinea
* Storage at 5°C

Basically, banana seeds are grouped into two types: barrel-shaped seeds in the *Callimusa* Section and subglobose or angular ones in the *Eumusa* Section (Figs. 1 and 2). Their colour ranges from pale brown to black. Seeds of the barrel type are 5-7 by 4-5 mm and the globose ones are 4-6 mm in diameter. The 1000-seed weight ranges from 38 to 94 g with the exception of seeds of *Ensete glauca,* which are very large and heavy with a 1000-seed weight of 600 g (Table 1). The basic structure of the seed is quite similar. At one end is the micropyle which is a plug-like structure. Behind it, the embryo is embedded in the endosperm and at the other end is the chalazal mass which, after drying, forms a cavity.

The anatomy of the seed can help to explain the germination and dormancy of the seeds. For example, *M. gracilis* has a cylindrical type of seed with a wide apical aperture at one end. A longitudinal section shows, below the aperture, the chalazal mass made up of thin-walled cells and, at the base, red-brown cells. The other half of the seed is filled with perisperm, the haustoria cotyledon and embryo and micropyle. The chalazal mass consists mainly of water; hence, freshly harvested seeds can germinate whereas, after drying, the tissue of the chalazal mass will shrivel and seeds become dormant. This helps to explain why banana seeds become dormant after drying (Kiew 1987).

Germination

Banana seeds of different species and varieties vary in size, shape and colour and require different conditions for germination. Some seeds germinate readily after harvesting and show no sign of seed dormancy; others become dormant after drying. Germination occurs in the first 2 months after sowing. In nature, banana seeds buried in the soil can remain viable for years and they germinate when soils are exposed, especially after the felling of forest trees.

In research studies, an attempt is made to study some of the factors affecting germination. Fruits are collected from the forests and seeds are extracted from mature ripe fruits. Seeds are cleaned and sown fresh or stored after being dried to 10-12% moisture content. In other studies pre-sowing treatments to break dormancy, such as scarification with sulphuric acid, chipping of testa, softening of testa by soaking and use of alternating temperatures, were used to promote germination.

A number of species of *Musa* were tested for their germination and a range of responses was encountered. Most of the species and varieties showed dormancy, except *Musa* or *E. glauca* which gave 100% germination without any treatment. All the seeds were sown in sterilized river sand. Moist freshly harvested seeds of *M. gracilis* of 46% moisture content resulted in 70% germination after moist heat treatment of 7 days. However, when seeds were dried to 12% moisture content and received heat treatment, there was no germination after 3 months. Seeds of *M. violascens*, when freshly harvested

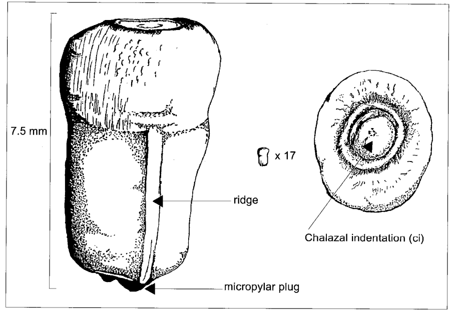


Fig. 1 (a). Left - Side view of *Musa violascens* seed; Right - Top view of *M. violascens* seed

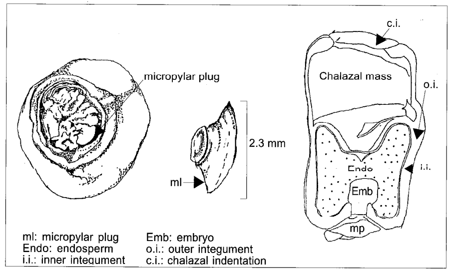


Fig. 1 (b). Left - Bottom view of *Musa violascens* seed; Centre - Side view of micropylar plug; Right - Longitudinal section of *M. violascens* seed

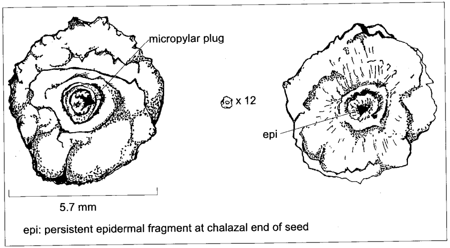


Fig. 2 (a). Seed of banana *Musa acuminata*. Left - Bottom view showing micropylar plug; Right - Top view of *M. acuminata* seed

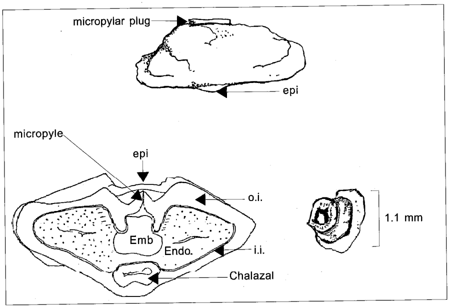


Fig. 2 (b). Top view of *Musa acuminata* seed; Bottom Left - Longitudinal section of *M. acuminata* seed; Bottom right- Micropylar plug

without drying and when sown in a glasshouse with ambient temperature, germinated readily within 21 days to give 96% germination. A sample of an unidentified species did not germinate when freshly harvested, but showed 65% germination after 3 months of storage. Thus, storing the seeds in this case breaks dormancy.

In general it is noted that the banana seeds show varying degrees of dormancy and they respond differently to various dormancy-breaking treatments. The most common and effective treatment is the use of alternating temperatures, i.e. germinating seeds under natural conditions in the glasshouse with day/night temperatures of 35-38/20°C. This can be better achieved in a germination or growth cabinet. As has been observed in the clearing of secondary jungles under disturbed soil, banana seedlings emerge readily. This is due to the exposure of seeds on the soil surface to alternating day and night temperatures.

It is best to harvest mature fruits and extract the hard black seeds, clean them and sow them in sterilized sand in a box at alternating temperatures of 35 and 20°C in a germinator or in a glasshouse under natural conditions. Seed viability can also be tested using 0.5% tetrazolium chloride solution; embryos of viable seeds will stain red.

Storage

Banana seeds are orthodox and hence can be dried to low moisture content and stored in subzero temperatures. Seed viability can easily be retained for 6 months after seeds have been sun-dried. According to Simmonds (1952), they can be stored for about 2 years without problem. The IBPGR advisory committee on *in vitro* storage at its second meeting (IBPGR 1984) stated that asexually propagated plants are more likely to retain somaclonal variations through successive generations than sexually propagated ones. For such plants, seeds can be used as a complementary storage method, especially for crops where there is little or no active breeding. For this research, the storability of seeds of wild banana species needs examination as a matter of urgency. In the meantime, genebanks can conserve germplasm as seed based on the results obtained so far.

Banana seeds can be dried from fresh seeds of moisture content around 45% to below 10%. This takes 3 days at 20°C. They can also tolerate very low temperatures, even down to -196°C, but their moisture content must be below 10%. Like all orthodox seeds, banana seeds have to be properly dried to low moisture content (below 10%, preferably 6-8%) for storage of 1-2 years. Even at ambient temperature, if seeds are kept in moisture-proof containers or in a desiccator, they maintain a high viability (>90% over a period of 2 years). At 10% moisture content, they remain viable for about 3 months in an air-conditioned room at 22°C. At lower temperatures, viability decreases if moisture content remains high.

A simple storage study was conducted in which the mature fruits of wild banana (*M. violascens*) were collected from the secondary forest reserves in Sungei Buloh, Selangor, Malaysia. After storage for up to 3 days at ambient room temperature (28 ±2°C), the fruits were processed. Skins were peeled off and the seeds extracted from the pulp and washed in water. The cleaned seeds were then dried at 22°C to different moisture levels, from an initial moisture content of 31% down to 29, 16 and 10%. Seed samples of three different moisture levels were sealed in plastic bags and vials for storage in liquid nitrogen. They were then placed at three different storage temperatures: 22, –20°C and –196°C.

For all these treatments of moisture levels and different storage, two replicates of 50 seeds each were used for storage periods of 1, 3, 6 and 12 months. For each treatment, seeds in plastic bags and vials for cryogenic storage were placed directly into the various temperature levels without precooling. After each storage period, germination tests were conducted by removing seeds from the plastic bags or vials and sowing them in sand in a box.

In the storage of orthodox banana seeds, moisture is the most important factor affecting storage. Those seeds with high moisture content decline in germination after 1 month of storage; seeds with 16 and 29% moisture content all failed to germinate by the third month in liquid nitrogen (Table 3). Although the percentage germination is rather low for all treatments, including the control, the fact that their viability was retained indicated that cold and

Table 3. The effect of moisture content and storage temperature on viability (%) of banana seeds after different storage times

Storage (months)	Moisture content[1]	Temperature[2]		
		T_1	T_2	T_3
1	M1	89	94	73
	M2	69	56	0
	M3	61	36	0
3	M1	66	68	70
	M2	56	54	0
	M3	47	17	0
6	M1	67	68	74
	M2	65	58	0
	M3	0	0	0
12	M1	50	41	44
	M2	35	31	0
	M3	0	0	0

[1] Moisture contents: M1 = 10%, M2 = 16% and M3 = 29%.

[2] Temperatures: T_1 = 22°C, T_2 = –20°C T_3 = –196°C.

cryogenic storage of banana seeds can be used at least for short-term storage and possibly for long terms also.

This study on the effect of various moisture levels and storage temperatures showed that banana seeds can be stored at low temperature. Further research is necessary on cryogenic storage of banana seeds, perhaps using lower moisture and progressive step-wise cooling instead of direct plunging. In theory, once the seeds can tolerate liquid nitrogen in storage, their viability should be maintained over long periods.

Conclusion

The seeds of 20 species, both varieties and wild banana, can be grouped into two types: (1) the cylindrical type, and (2) the subglobular, angular flattened type, but basically their structure and anatomy are very similar. A longitudinal section of both types shows that the chalazal mass fills up the upper section of the seed. Below the chalazal mass is the testa, separating it from the perisperm below; embedded in the basal part of the perisperm is the embryo and haustorium.

Fresh banana seeds that are high in moisture content germinate readily, but after drying they become dormant. The requirements for germination differ but, in general, an alternating temperature is preferred for germination. Properly dried banana seeds remain viable for a few months to 2 years. It is possible to use cryogenic storage for long-term genetic conservation.

References

Argent, G.C.G. 1976. The wild bananas of Papua New Guinea. Notes Roy. Bot. Gard. Edinb. 35:77-114.

Humphrey, J.E. 1986. The development of the seed in Scitamineae. Ann. Bot., Lond. O.S. 10:1-40.

IBPGR. 1978. Genetic resources of bananas and plantains. IBPGR, Rome, Italy.

IBPGR. 1984. Report of Advisory Committee on *in vitro* storage. IBPGR, Rome, Italy.

Jamaluddin, S.H. 1986. Characterization, evaluation and utilization of the banana germplasm in Malaysia. Pp. 315-329 *in* Proceedings Symposium Buah-buahan Keb. Serdang, Malaysia.

Kiew, R. 1987. Notes on the natural history of the Johore banana, *Musa gracilis* Holtum. Malayan Nature J. 41:239-248.

Simmonds, N.W. 1952. The germination of banana seeds. Trop. Agric. (Trinidad) 29:2-16.

Simmonds, N.W. 1953. Wild bananas in Malaya. Malayan Nature J. 10:1-8.

Tribe, D.E. 1994. Feeding and greening the world. The role of international agricultural research. CABI, Wallingford, Oxon, UK, 274p.

Annex 1

Importance of *in vitro* and *in vivo* collections in the world (INIBAP 1995)

Region	Country	Location of collection	No. of accessions
Asia/Pacific			
	Australia	UWA, Nedlands	10
		QDPI, Maroochy	368
		QDPI, South Johnstone	200
	Fiji	MAF, Koroniria	not known
	India	NBPGR, New Delhi	250
	Indonesia	BAU, Bogor	18
	Malaysia	MARDI, Serdang	79
	N.-Caledonia	CIRAD, Pocquereux	not known
	PNG	Laloki	>500
	Philippines	UPLB, Los Baños	170
		BPI, Davao	230
	Thailand	PHCR, Pichit	50
		M.A., Tachai	53
Latin America and the Caribbean			
	Brazil	IAC, Sao Paulo	not known
		IPAGRI, Itajai	103
		CNPMF, Cruz das Almas	300
	Colombia	CORPOICA, Agrado	>80
	Costa Rica	CATIE, Turrialba	>50
	Cuba	INIVIT, Villa Clara	277
	Guadeloupe	CIRAD, Neufchateau	>400
	Honduras	FHIA, La Lima	>400
	Jamaica	Banana Board, Kingston	350
	Nicaragua	ISCA, Managua	not known
Sub-Saharan Africa			
	Burundi	IRAZ, Gitega	271
	Cameroon	CRBP, Nyombe	500
	Ghana	Univ., Kade	not known
	Guinea	IRAG, Seredou macenta	not known
		Foulaya	not known
	Kenya	ICIPE, Mbita	167
		KARI, Kitale	not known
		KARI, Mtwapa Kikambala	19
	Nigeria	IITA, Onne	374
	Tanzania	TARO, Maruku	>300
	Uganda	NARO, Kawanda	342
		M.U.K, Kabanyolo	47

Annex 1 (continued)

Region	Country	Location of collection	No. of accessions
Other Regions			
	Belgium	INIBAP, ITC	>1000
	Spain	IRTA, Tenerife	not known
	USA	IFAS, Homestead	29
		U.G., Griffin	20