Collecting the *Musa* gene pool in Papua New Guinea

33

S. Sharrock
CARDI, University of West Indies, PO Box 64, Cave Hill Campus, St Michael, Barbados.

Introduction

Bananas were one of the earliest crops to be cultivated and both cooking and dessert bananas are still extremely important to the economies of many tropical countries. Though the dessert banana export industry is based on only one cultivar, many hundreds of varieties of bananas and plantains are cultivated worldwide as a subsistence food crop. Over the last few years there has been renewed interest in the collecting, maintenance and use of *Musa* germplasm, particularly as a source of disease resistance. This is due to recent disease outbreaks, such as that of race 4 of Panama disease (*Fusarium oxysporum* f. sp. *cubense*), to which Cavendish clones are susceptible, and the spread of Black Sigatoka disease (*Mycospherella fijiensis* var. *difformis*) into Africa.

At an international workshop held in Cairns, Australia, in 1986 it was recommended that further collecting should take place of the primary gene pool of *Musa* in Papua New Guinea, concentrating on wild material. The workshop also emphasized the dangers inherent in the movement of germplasm, particularly in relation to the banana bunchy top virus (BBTV). Though this is no longer the case, there was at that time no reliable indexing method for BBTV and plants had to be observed for visual symptoms of the disease over several months. In cooperation with the International Network for the Improvement of Bananas and Plantains (INIBAP), the Queensland Department of Primary Industries (QDPI) in Australia agreed to act as the quarantine centre for all *Musa* germplasm collected in Papua New Guinea.

Following the Cairns workshop, a *Musa* germplasm collecting project was initiated in 1987 with the following aims:

- to collect indigenous banana cultivars and wild *Musa* species in Papua New Guinea;
- to gain a better knowledge of the genetic diversity within the wild species;
- to tissue-culture and disease-index the collected germplasm;
- to supply INIBAP with disease-free germplasm for long-term storage and for distribution to banana gene banks and to improvement programmes, including, of course, that of Papua New Guinea.

Planning the germplasm collecting mission

In order to obtain the maximum amount of information in the field, a multidisciplinary team was involved in each mission, typically including a banana taxonomist, a plant pathologist with particular experience of bananas and a person with local knowledge. The selection and coordination of the collecting team was an essential part of the planning process, which involved addressing the issues of:

- which species to collect;
- which areas to visit;
- how to collect and maintain the germplasm.

Target taxa

An understanding of the taxonomy of the target taxa and knowledge of their distribution are essential before undertaking any collecting work. The taxonomy of *Musa* is complicated, but there are numerous works both on the genus as a whole and on its representatives in different regions, including Papua New Guinea (Cheeseman, 1947–50; Simmonds, 1956, 1962; Argent, 1976; Stover and Simmonds, 1987; Shepherd, 1990; Simmonds and Weatherup, 1990).

The family Musaceae comprises two genera, *Ensete* and *Musa*. *Ensete* is considered to be an old genus which probably originated in Asia, spreading from there to Africa (Purseglove, 1985). It consists of six or seven species, which are equally divided between the two continents. One species (*E. glaucum*) is widely distributed through southeast Asia and is found in Papua New Guinea. The genus *Musa* contains approximately 40 species distributed throughout southeast Asia and the Pacific. The centre of diversity and probably of origin of the genus is the Assam–Myanmar–Thailand area (Simmonds, 1962). *Musa* is divided into four sections: *Eumusa*, *Australimusa*, *Callimusa* and *Rhodochlamys*.

Eumusa ($2n = 2x = 22$) is the largest and most diverse section of *Musa*, being distributed from southern India to Japan and Samoa. Almost all edible, cultivated bananas are in this group. Three species are found in Papua New Guinea (*M. acuminata* ssp. *banksii*, *M. schizocarpa* and *M. balbisana*). The section *Australimusa* ($2n = 2x = 20$) contains six or seven

species distributed from Queensland in Australia to the Philippines. The Fe'i group of edible bananas (related to *M. maclayi*) belongs here. *M. textilis* (abaca or Manila hemp) is also included in this group. Five sect. *Australimusa* species are found in Papua New Guinea (*M. maclayi*, *M. boman*, *M. peekelii*, *M. bukensis* and *M. lolodensis*).

One other *Musa* species is present in Papua New Guinea. This is *M. ingens*, which has a chromosome number of $2n = 2x = 14$ and is reputed to be the largest known herb (Purseglove, 1985). It was placed in a new section (sect. *Ingentimusa*) by Argent (1976), the validity of which has been questioned by Simmonds and Weatherup (1990). No species from the sections *Callimusa* ($2n = 2x = 20$) and *Rhodochlamys* ($2n = 2x = 22$) have been found in Papua New Guinea. They are distributed from Indochina to Malaysia and from India to Indonesia respectively.

All edible bananas (with the exception of the Fe'i bananas) are derived from *M. acuminata* (A genome) and *M. balbisana* (B genome) in the section *Eumusa*. The centre of diversity of *M. acuminata* is the Malaysia–Thailand area, where four of the five currently recognized subspecies overlap. It is considered that wild-seeded forms of this species may have been used by early fishermen, who used the leaves and leaf-sheaths for fibre and as wrapping material and plates and who may have eaten the male buds and immature fruits. Edibility of the mature fruit of diploid *M. acuminata* came about as a result of two important changes, parthenocarpy and female sterility. As a result of the former, fruit pulp develops without the stimulus of pollination, but should pollination occur the latter ensures that seeds are not formed. These characters would have been deliberately selected for by humans and maintained by vegetative propagation.

Early cultivated bananas were diploids entirely derived from *M. acuminata* and are called AA diploids. Triploid *M. acuminata* (AAA; $2n = 33$) forms also appeared, apparently as a result of hybridizations in which partly sterile edible diploids crossed with male-fertile forms. Such triploids contain 22 chromosomes (AA) from the female gamete and 11 chromosomes (A) from the haploid pollen. Such triploid plants have larger fruits and are more vigorous, more productive and hardier than the diploids. They were selected in preference to the diploids, which they replaced in most areas. One exception to this is Papua New Guinea, where, due to its ancient isolation, AA diploids remain agriculturally important and AAA triploids are less common.

Diploid (AA) and triploid (AAA) cultivars were taken by humans to areas where the wild species *M. balbisiana* was native. In these areas natural hybridizations took place to produce progeny with the genomes AB, AAB and ABB. No edible diploid (BB) form of *M. balbisiana* is known but it is thought that triploid BBB forms may be present in the Philippines (Vakili, 1967; Valmayor *et al.*, 1981). *M. balbisiana* is indigenous to areas with a monsoon climate and a pronounced dry season. The B genome confers drought resistance and hardiness to the diploid

and triploid hybrids as well as introducing variation in disease resistance and fruit quality.

Bananas are one of the most important food crops in Papua New Guinea. They are grown throughout the country, including the highlands, up to an altitude of 2000 m above sea level. As mentioned above, the unique feature of banana cultivation in Papua New Guinea is the importance of AA diploids. It is estimated that there may be as many different diploid clones growing there as in the whole of the rest of the world (Stover and Simmonds, 1987). The present situation in Papua New Guinea is considered to resemble that of Malaysia thousands of years ago, when poor-yielding, primitive diploid cultivars predominated. They have now been replaced by more vigorous and productive triploid (AAA) clones.

Banana and plantain improvement programmes are, on the whole, aimed at improving the production of either dessert bananas (AAA triploids) or plantain-type cooking bananas (AAB triploids). Most triploid AAA and AAB clones, however, are almost completely sterile, making banana breeding particularly difficult. Diploid cultivars, being frequently male-fertile and sometimes also female-fertile, are thus of particular importance. If economic pressures should cause changes in traditional farming practices in Papua New Guinea, the diploids may be lost; hence the urgent need for conservation.

The disease Black Sigatoka (*Mycospherella fijiensis* var. *difformis*) is also widespread in Papua New Guinea; indeed, the region including that country and the Solomon Islands is believed by some to be the centre of origin of the fungus (Stover, 1978). If this is the case, Black Sigatoka may have been exerting selection pressure as the predominant leaf spot for a considerable period, resulting in some level of resistance in indigenous cultivars. During the planning stages of the collecting work it was therefore decided to put emphasis on collecting indigenous diploid rather than triploid cultivars.

A national banana germplasm collection was established in Papua New Guinea in the early 1970s. Before carrying out any collecting work a visit was made to this field collection to ascertain how much diversity was already represented in the gene bank. The original collection contained 675 accessions but this had declined to 195 by 1988 (Sharrock, 1990). Most of the accessions that were lost during this period were AA diploids and wild species. Triploids AAA, AAB and ABB were well represented in the collection, which also contained several possible tetraploids. Only two wild species were present (*M. maclayi* and *M. balbisiana*). The visit to this germplasm collection confirmed that, to avoid duplication, the project should concentrate on collecting AA diploid cultivars and the wild *Musa* species.

Target areas

It was first necessary to identify those specific areas where AA diploids were known to be agriculturally important. Two areas, East New Britain

and Madang, were initially selected on the basis of published accounts (Stover and Simmonds, 1987), information from the national banana germplasm collection (Shepherd and Ferreira, 1984) and consultations with local agricultural experts. These areas were covered during the first two collecting missions. A range of ecogeographical zones were then visited during subsequent visits. The identification of potential germplasm collecting sites was made in collaboration with local Department of Agriculture staff.

A comprehensive study of the wild bananas of Papua New Guinea was carried out by Argent (1976). This includes information on the distribution of each species. In order to ensure that germplasm of all the wild species was collected, areas where each species was known to be present were visited, as well as some areas not previously covered.

Germplasm collecting and movement

Banana cultivars are vegetatively propagated and have to be collected as suckers. The dangers of moving germplasm in this form are well recognized and guidelines for the safe movement of *Musa* germplasm have been published (Frison and Putter, 1989). For the purposes of this project, the QDPI agreed to provide the facilities for the tissue culturing and disease screening in quarantine of the collected germplasm. The following germplasm introduction procedure was developed:

1. Plants were imported as deleafed suckers and meristems were established in tissue culture to eliminate risks from fungal, bacterial and nematode pathogens.
2. Representative plantlets from each tissue-cultured meristem were grown in the quarantine glasshouse for visual screening for symptoms of BBTV and disease indexing for cucumber mosaic virus (CMV).
3. If screened plants were deemed free of disease, all material derived from that meristem was released from quarantine and *in vitro* cultures were sent to INIBAP.

Banana suckers can only be stored for a limited period of time after collecting, particularly in hot, humid conditions. To overcome this problem, it was decided that the collecting work should be carried out in a series of short missions, each one lasting for no longer than one month, so that suckers could be returned to the tissue culture laboratory while still in good condition.

Wild species can be collected as seed rather than suckers and are for this reason easier to deal with from a quarantine point of view. Seed samples are also easier to carry and store during collecting missions and it was decided to collect wild species as seed whenever possible. No bacterial or fungal pathogens are known to be seed-borne in *Musa*, but the situation regarding virus diseases is less clear. It is possible that CMV may be seed-borne (Gold, 1972) and all plants grown from seeds were therefore indexed for this disease. There are no reports to date of BBTV or any other virus disease being transmitted via seed.

In the field

Wild bananas typically appear as early colonizers of cleared forests. Roadsides and the edges of cultivated land are thus ideal habitats, making these species relatively easy to find and collect. The only exception is *M. ingens*, which grows not only at a higher altitude than any other *Musa* species but also well within forested areas rather than on their edges.

Slightly different sampling strategies must be used depending on the breeding system of the species involved. Species that were known to be self-pollinating (i.e. those that have hermaphrodite basal flowers, such as *M. schizocarpa*) could be safely collected as seed as they breed true to type. Populations of such species consist of individuals which are more or less homozygous. Seeds from any one individual will give progeny that are very similar to each other and to the parent plant. To collect maximum diversity, small seed samples were taken from a number of individuals rather than large samples from any one plant. Many *Musa* species are cross-pollinated, however, with their basal 'hands' functionally female. Species such as *M. balbisiana* will produce variable progeny when grown from seed. Therefore, if a particular plant was seen with a desirable or uncommon characteristic, suckers were collected in addition to a population sample of seed, so as to ensure the preservation of that particular genotype.

Where the ranges of two species overlap, hybrids may be formed, as is the case with *M. acuminata* ssp. *banksii* and *M. schizocarpa* in Papua New Guinea. Such hybrids are generally sterile, producing poorly developed seed, and had to be collected as suckers. However, embryo culture techniques can be used to rescue embryos from poorly developed seeds which would not normally germinate, so both seeds and suckers were collected to enable the parent and its offspring to be compared during characterization. In areas where hybrids grew, care was taken when collecting each parent species to ensure that seeds were taken from plants well isolated from the other parent, so as to avoid collecting only seeds of hybrid origin.

While wild species can be collected as either seeds or suckers, cultivated types have to be collected as suckers. A good starting place was often the local market. Here it was possible to form an idea of the range of varieties being grown in the area, and discussions with local producers helped to identify places where these cultivars could be collected. The assistance of provincial Department of Agriculture extension officers was often essential, as they had extensive knowledge of the area and were able to arrange visits to farmers known to cultivate a range of varieties. Farmers were also more likely to cooperate in donating suckers if the collecting team included someone they knew.

One of the main difficulties in collecting cultivated *Musa* is distinguishing diploids and triploids. There are, of course, published identification aids, but including an experienced banana taxonomist in the

collecting team mitigated this problem. The recognition of synonyms of vernacular names is more complicated. In a country such as Papua New Guinea, which has as many as 700 different languages, the local name for the same banana cultivar often changes from one village to the next. When this is compounded by phenotypic variation due to differences in growing environments and with the local traditions of covering bunches and removing male buds, it is easy for duplicates to be collected. In an attempt to minimize this, detailed descriptions were made and photographs taken of the plant as it was collected. These were then used in subsequent collecting missions. The knowledge of local banana varieties of farmers and local agricultural extension staff also proved useful in avoiding collecting duplicates.

Handling and care of collected material

Suckers were collected only from apparently healthy plants, with a minimum of damage being inflicted on the parent plant. Suckers had to be at least 10 cm in diameter at the base and ideally with little or no weevil borer (*Cosmopolites sordidus*) damage to the corm. Larger suckers were cut back before transportation but smaller suckers were avoided unless the period of storage was to be no more than a few days. Whenever possible, at least three suckers were collected per plant.

Once removed from the parent plant, the sucker was labelled and cleaned and any damaged outer layers of tissue were removed. The upper portion of the sucker was cut back to approximately 20 cm above the meristem and the corm trimmed to approximately 10 cm below the meristem. The cleaned and trimmed sucker was then allowed to dry before being wrapped in newspaper for storage. Suckers prepared in this way could be stored for up to three weeks, or longer if kept in cool, dry conditions.

One of the main problems encountered in the storage of suckers was rotting due to weevil borer damage. It was frequently difficult to obtain clean suckers and often what appeared to be relatively minor damage on the outside of the sucker was actually considerably worse towards the centre of the corm. Because of this, whenever possible during the collecting missions suckers were sent back to the tissue culture laboratory by air freight so as to minimize the period of storage.

Banana fruits contain from 30 to more than 150 seeds each, which must be mature (i.e. hard and black) when collected. The seeds mature before the fruit, so immature fruit may contain seeds suitable for collecting. Seeds are easier to store and carry than suckers and can be kept fresh inside the fruit after collecting if desired. Banana seeds exhibit dormancy and, although they germinate readily when freshly harvested, if dried they may take two to six months to germinate. If seeds had to be extracted from the fruit before returning to base, they were carefully cleaned, which was most easily done when the fruits were very ripe. The

seeds were then kept moist if the period of storage was to be no more than one or two weeks. If a longer period of storage is required, seeds must be dried. The moisture content of banana seeds can be reduced to around 10% by drying at 20°C for three days in an air-conditioned room. At this moisture content they will remain viable for about three months at an ambient air temperature of around 22°C. For longer-term storage, the moisture content and temperature of storage must be reduced (see below).

It is possible in bananas to use the meristem contained within the male bud as an explant for initiating tissue cultures. In situations where neither seeds nor suckers were available, the male bud was therefore collected. It is not possible to store the male bud for more than a few days, but if it could be returned to the tissue culture laboratory within that time it was a viable alternative to suckers.

Data collecting

Data collected in the field were of crucial importance, particularly for the identification of duplicates, as already mentioned. Preprinted forms were taken to the field to record details of each sample. In addition to recording the usual passport data (e.g. location, altitude, collecting date, etc.), descriptions were made of the plant as a whole (size, colour, amount of suckering) and of the bunches (number of hands, angle of bunch, size of fruit; presence/absence, size, colour and shape of male bud; colour of male flower and presence of pollen). Distinctive features and the presence (or absence) of pests and diseases were also recorded. Local names and uses were documented from interviews with farmers.

This was not considered a definitive description of the material and absence of a disease or pest that was locally prevalent was not taken as necessarily indicative of resistance. Growing conditions can have a significant effect on the expression of many characters in bananas, and detailed descriptions would be carried out later, with the plants growing under experimental conditions.

Back at base

On arriving back at base the suckers were established in tissue culture as soon as possible. Full details of the methods used in the shoot-tip culture of *Musa* are given elsewhere (Cronauer and Krikorian, 1984; Vulysteke, 1989). Many accessions collected during the first mission were lost due to contamination of the cultures. As a result, a double surface-sterilization procedure was developed. The culture medium used was a basic Murashige and Skoog (1962) medium supplemented with 2.5 mg l^{-1} 6-benzylaminopurine, 20 g l^{-1} sucrose and 8 g l^{-1} agar.

During the first few weeks in culture, blackening sometimes occurs

at the cut surfaces of the corm due to the oxidation of phenolic compounds in the wounded tissues. The extent of blackening varies with species and cultivar but it is severe in cultures of *M. balbisiana* and ABB cultivars. It was also found to be a problem with the sect. *Australimusa* species and Fe'i cultivars. When blackening occurred the blackened tissue had to be cut away and the explant transferred to fresh medium regularly, possibly every few days, until the blackening was reduced. If blackening was particularly severe explants were pretreated by immersion in a sterile solution of an antioxidant (e.g. cysteine, ascorbic acid or citric acid) prior to inoculation in the medium, or an antioxidant was added to the medium (Vuylesteke, 1989).

The method for initiating shoot-tip cultures recommends a double surface-sterilization treatment and a relatively large final explant (approximately 1 × 2 cm). The reason for this was that frequently only one or two suckers per accession were available and losses due to contamination in culture had to be minimized. Having a large explant meant that, should contamination occur, the explant could be subjected to a further surface-sterilization treatment. Secondly, if blackening occurred it was possible to remove the outer blackened layers of tissue without damage to the meristem.

Seeds were dried in preparation for storage and a sample germinated for characterization. If seeds were brought back to base in the fruit, they were cleaned before drying. Because the base was outside the country of collecting, the fruit was under quarantine restrictions due to the possibility of introducing foreign types of fruit fly. To prevent this happening the fruit was immersed in an insecticide solution before the pulp was removed from the seeds. After cleaning, seeds were dried, ideally to 6–8% moisture content, which can be done at 15°C and 15% relative humidity or in an air-conditioned room at 20°C.

Seeds could then be stored at subzero temperatures in sealed, vapour-proof containers (Kar Ling Tao, pers. comm.). The viability of banana seeds in long-term storage is being investigated by Professor Chin at the University Pertanian in Malaysia. It is known that they can remain viable for at least one to two years under the above conditions and cryogenic storage of seeds or embryos may be a possibility in the future. Fresh seeds will germinate readily, but after drying the seeds become dormant and may take two to six months to germinate at ambient temperatures. However, seeds exposed to alternating temperatures of 35/20°C germinate more readily.

Embryo cultures were mainly used to 'rescue' embryos of hybrid origin which would not germinate under normal conditions, but tissue cultures of accessions with viable seeds were also required. Banana seeds are small, generally 4–6 mm in diameter, although size does vary with species, and the outer layer is very hard. The structure of seeds is similar in all species. At one end of the seed is the micropyle, below which lies the embryo, embedded in the endosperm. Below the embryo, at the other end of the seed is the chalazal mass. For embryo culture, the seeds were

split open without damaging the embryos, which were then removed and placed on the culture medium using sterile techniques. The embryos, which are less than 1 mm in length, were removed with the help of a stereomicroscope. The medium used for embryo culture was the same as that used for shoot-tip culture. Germination of the embryos usually took place within one to two weeks. Cultures originating from different seeds were labelled separately, even though the seeds may have originated from the same plant, to take account of variation within the seed lot. More details of the embryo culture of bananas are given by Escalant and Teisson (1987).

Report writing

After each collecting mission a report was written giving background information on the collecting mission and details of what was collected and where. A map indicating the germplasm collecting sites and the distribution of the wild species collected was always included. A summary of the diversity in different areas was provided. The reports also highlighted the incidence and severity of pests and diseases encountered. Brief details of each sample were included as an appendix to the report. An example of a report written after a *Musa* collecting mission to Papua New Guinea is given by Sharrock (1990). Reports were circulated to the International Board for Plant Genetic Resources (IBPGR) and INIBAP as well as to other interested parties both within Papua New Guinea and elsewhere.

Results of the collecting work

A total of four collecting missions were made to Papua New Guinea during 1988–89. Two hundred and sixty accessions were collected, of which 116 have been tentatively classified as AA diploids, 51 accessions came from nine of the ten wild species present in Papua New Guinea and the remaining 93 accessions comprise a mixture of triploids, tetraploids and Fe'i cultivars. All these accessions have been established in tissue culture and representative plantlets screened for diseases in quarantine. Cultures of disease-free accessions have also been sent to the *in vitro* laboratory of INIBAP for storage and distribution, including return to Papua New Guinea for inclusion in the national banana germplasm collection.

Triploid clones, many introduced from other countries, are found growing close to houses and along roadsides and are not frequently replanted. In contrast, diploid bananas are planted in mixed food gardens, which may be located some distance from houses. These food gardens are replanted after each harvest and the diploid bananas are as a result treated more or less as an annual crop. This system does not

allow diseases and pests to build up. The greatest diversity in diploid bananas is to be found in areas such as East New Britain, Madang and Kiunga in Western Province, where bananas form the staple food crop. In seasonally dry areas banana production is based on triploid ABB cultivars, which are more drought resistant than AA diploids. In the highland areas most of the banana cultivars are AAB triploids, unusual in their erect leaf habit.

The most widely distributed wild *Musa* species in Papua New Guinea are *M. acuminata* ssp. *banksii* and *M. schizocarpa*. These are probably the ancestral parents of many of the traditional Papua New Guinea cultivars. Variation within these species is limited, although some differences in bract colour and finger shape do occur. Natural hybridization between the species does occur but no signs of introgression with either parent have been observed. A few unknown hybrids were collected. These may have originated from crosses between wild species and cultivated diploids, the most likely parents being a male-fertile diploid and *M. acuminata* subsp. *banksii*, which is known to sometimes lack functional stamens in its basal hands and does hybridize with *M. schizocarpa*. Such hybrids were rare even in areas where wild species were growing in close proximity to cultivated diploids.

Five species from sect. *Australimusa* are found in Papua New Guinea (*M. maclayi*, *M. peekelii*, *M. lolodensis*, *M. boman* and *M. bukensis*). All of these were collected except *M. bukensis*, which is only recorded from the island of Bougainville, where, due to the political situation at that time, it was not possible to collect. A number of Fe'i cultivars were collected from various locations throughout Papua New Guinea. These are not a popular food source but are kept as a backup for when other food is scarce. They tend to be vigorous plants resistant to disease and require little attention. *M. ingens* and *Ensete glaucum* were also collected.

References

Argent, G.C.G. (1976) The wild bananas of Papua New Guinea. *Notes from the Royal Botanic Garden Edinburgh* 35:77–114.

Cheeseman, E.E. (1947–50) The classification of the bananas. *Kew Bulletin* 1947:97–117; 1948:11–28, 145–157, 323–328; 1949:23–28, 133–137, 265–272, 445–452; 1950:27–31, 151–155.

Cronauer, S.S. and A.D. Krikorian (1984) Multiplication of *Musa* from excised stem tips. *Annals of Botany* 53:321–328.

Escalant, J.V. and C. Teisson (1987) Comportements *in-vitro* de l'embryon isolé du bananier (*Musa* species). *Fruits* 42:33–342.

Frison, E.A. and C.A.J. Putter (eds) (1989) *FAO/IBPGR Technical Guidelines for the Safe Movement of* Musa *Germplasm*. FAO/IBPGR, Rome.

Gold, A.H. (1972) Seed transmission of banana viruses. *Phytopathology* 62:760.

Murashige, T. and F. Skoog (1962) A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiologia Plantarum* 15:473–497.

Purseglove, J.W. (1985) *Tropical Crops - Monocotyledons*. Longman, London.

Sharrock S.L. (1990) Collecting *Musa* in Papua New Guinea. In: Jarret, R.L. (ed.) *Identification of Genetic Diversity in the Genus* Musa. Workshop Proceedings. 5-10 September 1988. Los Baños, Philippines. pp. 140-157. IRRI, Los Baños.

Shepherd, K. (1990) Observations on *Musa* taxonomy. In: Jarret, R.L. (ed.) *Identification of Genetic Diversity in the Genus* Musa. Workshop Proceedings. 5-10 September 1988. Los Baños, Philippines. pp. 158-165. IRRI, Los Baños.

Shepherd, K. and F.R. Ferreira (1984) The Papua New Guinea Biological Foundation's banana collection at Laloki, Port Moresby, Papua New Guinea. *IBPGR/ Southeast Asia Newsletter* 8:28-34.

Simmonds, N.W. (1954) Notes on banana varieties in Hawaii. *Pacific Science* 8:226-229.

Simmonds, N.W. (1956) Botanical results of the banana collecting expedition, 1954-5. *Kew Bulletin* 1956:463-489.

Simmonds, N.W. (1962) *The Evolution of the Bananas*. Longman, London.

Simmonds N.W. and T.C. Weatherup (1990) Numerical taxonomy of the wild bananas (*Musa*). *New Phytologist* 115:567-571.

Stover, R.H. (1978) The distribution and probable origin of *Mycosphaerella fijiensis* in South East Asia. *Tropical Agriculture (Trinidad)* 55:65-68.

Stover, R.H. and N.W. Simmonds (1987) *Bananas*. Longman, London.

Vakili, N.G. (1967) The experimental formation of polyploidy and its effect in the genus *Musa*. *American Journal of Botany* 54:24-36.

Valmayor, R.V., F.N. Rivera and F.M. Lomuljo (1981) *Philippine Banana Cultivar Names and Synonyms*. IPB Bulletin No. 3. National Plant Genetic Resources Laboratory, Institute of Plant Breeding, University of the Philippines, Los Baños.

Vuylysteke, D.R. (1989) *Shoot Tip Culture for the Propagation, Conservation and Exchange of* Musa *Germplasm*. IITA, Ibadan, Nigeria.