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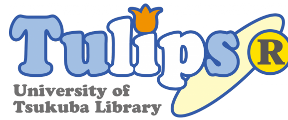

Assessment of Genetic Diversity among the Major Myanmar Banana Landraces

著者	Wan Yusong, Watanabe Junko, Yi San San, Htaik Than, Win Kyaw, Yamanaka Shinsuke, Nakamura Ikuo, Watanabe Kazuo
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Note

Assessment of Genetic Diversity among the Major Myanmar Banana Landraces

Yusong Wan*[1,2], Junko A. Watanabe[1], San San Yi[3], Than Htaik[3], Kyaw Win[3], Shinsuke Yamanaka[1], Ikuo Nakamura[4] and Kazuo N. Watanabe[1]

[1] *Gene Research Center, Graduate School of Life and Environmental Sciences, University of Tsukuba*, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan

[2] *Biotechnology Research Institute, Chinese Academy of Agricultural Sciences*, 12 Zhongguancun South Street, Beijing 100081, China

[3] *Vegetable and Fruit Research and Development Center, Myanmar Agriculture Service, Ministry of Agriculture and Irrigation*, Myanmar

[4] *Graduate School of Science and Technology, Chiba University*, 648 Matsudo, Matsudo, Chiba 271-0092, Japan

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Bananas (*Musa* spp.), which originated in South East Asia and the western Pacific area and were then introduced into other continents (Simmonds 1966), are a staple food for hundreds of millions of people living in tropical countries. Worldwide production of banana crops has now reached 95 million tons per year (FAO 2002) and in the developing world, the banana is ranked as the fourth most important staple food crop (after rice, wheat and maize). However, despite its socioeconomic importance, banana breeding has been unsuccessful for many decades. This has mainly been due to the existence of polyploidy, sterility and parthenocarpy. However, for the past 10 years, *Musa* researchers worldwide have recorded a number of important achievements.

The genus *Musa* L. comprises about 30 to 40 species of perennial rhizomatous herbs (Simmonds 1995). The characterization and classification of *Musa* germplasm are of paramount importance, not only to taxonomists but also to plant breeders. *Musa* species were first classified by morphological characteristics and the formal classification of the genus *Musa* was first developed by Baker (1893) and then revised by Cheesman (1947), who divided the genus into four sections: *Eumusa*, *Rhodochlamys*, *Australimusa* and *Callimusa*. Cheesman's classification provided a general framework for future studies on *Musa* taxonomy and was used for almost half a century without any significant modifications (De Langhe 2000). Furthermore, the *Eumusa* species of banana, which include modern cultivars, appear to be very diverse (Nwakanma *et al.* 2003). Modern cultivated bananas, which have mostly triploid genomes, evolved from intra- and interspecific hybridization between two wild diploid species of *Eumusa*, *M. acuminata* Colla. and *M. balbisiana* Colla., that contributed the A and B genomes, respectively (Simmonds 1995). Further evolution occurred through polyploidization and the accumulation of somatic mutations (Stover and Simmonds 1987). The most popular system of classification of *Musa* cultivars was developed by Simmonds and Shepherd (1955), based on 15 morphologic diagnostic characteristics (or descriptors). Ortiz *et al.* (1998) also developed a phenotypic diversity index based on 16 quantitative descriptors for the classification of *Musa* cultivars from Africa.

Plant morphology is often considerably affected by the environment, and for a consistent classification of hybrids, their evaluation in different environments is necessary. In addition, if some characteristics cannot be scored, these determinations will necessarily be incomplete (Perrier and du Montcel 1990). Molecular markers, which have advantageous attributes in this regard, are widely used in the determination of *Musa* genome composition and genetic diversity studies, and have dramatically improved *Musa* breeding efficiency (Tenkouano *et al.* 1999a, 1999b). Howell *et al.* (1994) used RAPD markers to classify nine *Musa* genotypes into four groups, representing the AA, AAA, AAB and BB genomes. Furthermore, Pillay *et al.* (2000) also identified RAPD markers linked to A and B genome-sequences in *Musa.* Umali and Nakamura (2003) reported a single nucleotide polymorphic sequence (SNP) in the *trnL-F* intergenic spacer region of chloroplast DNA, that discriminates between *M. acuminata* (AA) cytoplasm from *M. balbisiana* (BB) cytoplasm, and used it to generate dCAPS (derived cleaved amplified polymorphic sequence) markers. Nwakanma *et al.* (2003) also developed an PCR-RFLP marker system from ribosomal DNA internal transcribed spacers (ITS) for discriminating between the A and B genomes of *Musa*. Ude *et al.* (2002a, 2002b) have analyzed the genetic diversity and relationships among different sections and species of *Musa* using AFLP marker sets and Jin *et al.* (2000) have also reported the use of AFLP markers to analyze polymorphisms in banana cultivars. Creste *et al.* (2003) have reported the use of microsatellite markers in their studies on

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*Corresponding author (e-mail: wanyusong@hotmail.com)

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the genetic characterization of banana cultivars from Brazil, while Onguso *et al.* (2004) used RAPD markers to analyze the genetic relationships between Kenyan banana cultivars. Umali *et al.* (2002) have developed molecular markers from plastid-subtype identity (PS-ID) sequences for use in DNA fingerprinting analysis which enables to discriminate between *Musa* AAA Cavendish subgroup cultivars. Some markers related to disease expression have also been identified (Lheureux *et al.* 2003) and a molecular map of the A genome species is presently available (Faure *et al.* 1993). Ortiz (1997) had previously proposed a crop-breeding strategy based on the domestication of banana and plantain species and molecular marker technology has been shown to considerably improve the breeding efficiency in *Musa* (Tenkouano *et al.* 1999a, 1999b).

Myanmar displays a high diversity of *Musa* species and has many landraces with unique skin colors. In the present study, we were interested in the diversity of landraces in Myanmar, to determine how they differ from international cultivars and whether Myanmar landraces with the same original name are in fact identical clones or belong to different genotypes. In our experimental design, 13 banana landraces from different regions of Myanmar (provided by the Vegetable and Fruit Research and Development Center, VFRDC), which were unclassified materials of local importance and could be identified by their original names, were used to detect polymorphisms. Eleven international standard cultivars, supplied by the International Network for the Improvement of Banana and Plantain (INIBAP), were analyzed in the same way for comparison (Table 1). Total DNA isolates were extracted from the leaves of each sample, according to the procedure of Gawel and Jarret (1991) with some modifications.

P450-based analog (PBA) markers, which are based on a specific multi-gene family and which we developed for PCR amplification of 15 marker pairs using 8 specific primers (Yamanaka *et al.* 2003), were used to detect polymorphisms in the banana landraces and cultivars in the present study. The primer-sets were composed of three forward primers (CYP1A1F, CYP2B6F and CYP2C19F) and five reverse primers (CYP1A1R, CYP2B6R, CYP2C19R, heme2B6 and heme2C19) (Table 2). PCR amplification was performed using 20 ng of extracted DNA in a total reaction mixture of 25 μl containing 1 × PCR buffer (Takara), 0.16 mM of dNTPs, 1 mM of each primer and 1 unit of *Taq* polymerase (Takara). A reaction of 32 cycles was programmed for 1 min at 94°C, 2 min at 45°C and 3 min at 72°C in a Thermal Cycler (MJ PTC-200). Annealing temperatures for each primer-set were adjusted according to the method of

Table 1. Banana landraces and cultivars used in the present study

Accession No.	Original name	Skin color	Origin
MS2-1	Thanda sein	Green	Myanmar
MS2-2	Thanda sein	Green	Myanmar
MS4-2	Low BNN-1	Yellow	Myanmar
MS5-1	Shwe Ni	Red	Myanmar
MS8-2	Wet Ma Lot	Yellow	Myanmar
MS9-1	Khin Wa	Yellow	Myanmar
MS10-1	Khin Wa-2	Yellow	Myanmar
MS10-2	Khin Wa-2	Yellow	Myanmar
MS11-1	Yatana	Yellow	Myanmar
MS11-2	Yatana	Yellow	Myanmar
MS13-2	Ind	Yellow	Myanmar
MS17-1	LKB-II	Yellow	Myanmar
MS19-2	Khun War II	Yellow	Myanmar
STC02	Giant Cavendish	Yellow	INIBAP
STC03	Umalag	Yellow	INIBAP
STC06	–	Yellow	INIBAP
STC13	–	Yellow	INIBAP
STC15	–	Yellow	INIBAP
STC29	Lakatan	Yellow	INIBAP
STC33	Orito	Yellow	INIBAP
STC36	Tanduk	Yellow	INIBAP
STC37	–	Yellow	INIBAP
STC51	Cardava	Yellow	INIBAP
STC52	Saba#1	Yellow	INIBAP

Table 2. PBA primers used in the present study

Primer	Sequence (5′ to 3′)
CYP1A1F	GCC AAG CTT TCT AAC AAT GC
CYP2B6F	GAC TCT TGC TAC TCC TGG TT
CYP2C19F	TCC TTG TGC TCT GTC TCT CA
CYP1A1R	AAG GAC ATG CTC TGA CCA TT
CYP2B6R	CGA ATA CAG AGC TGA TGA GT
CYP2C19R	CCA TCG ATT CTT GGT GTT CT
heme2B6	ACC AAG ACA AAT CCG CTT CCC
heme2C19	TCC CAC ACA AAT CCG TTT TCC

Table 3. Analysis of genetic diversity in banana specimens using PBA markers

Primer sets	Genetic diversity[1]		
	Myanmar landraces (n=13)	International standard cultivars (n=11)	All (n=24)
CYP1A1F/CYP1A1R	0.363	0.485	0.435
CYP1A1F/CYP2C19R	0.325	0.307	0.366
CYP1A1F/heme2B6	0.281	0.217	0.298
CYP2B6F/CYP2B6R	0.292	0.422	0.389
CYP2B6F/heme2B6	0.343	0.248	0.326
Average	0.321	0.336	0.363

[1] From Nei (1973)

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Yamanaka *et al.* (2003). PCR products were then electrophoresed on 1% agarose gels.

Results were scored as the presence or absence of amplified fragments. Genetic similarities between banana landraces and cultivars were calculated based on the Jaccard (1908) coefficient. A dendrogram was then constructed using the unweighted pair-group method of the arithmetic average (UPGMA). Principal component analysis (PCA) was also carried out to reveal the multiple dimensions of the distribution of both landraces and cultivars within a scatter-plot.

For the 13 banana landraces from Myanmar, six pairs of primers generated 46 bands that could be scored, including 30 polymorphic bands. The frequency of polymorphisms was 65.2%. For the 11 international standard cultivars supplied by INIBAP, five pairs of primers generated a total of 39 bands that could be scored, including 26 polymorphic bands at a frequency of 66.7%. For all the materials, five pairs of primers yielded 42 bands that could be scored, including 27 polymorphic bands at a frequency of 64.3%. The average gene diversity calculations (Nei 1973) for the Myanmar landraces and international standard cultivars were 0.321 and 0.336, respectively and the average genetic diversity of all the analyzed materials was calculated at 0.363 (Table 3).

UPGMA clustering analysis showed that the 13 landraces obtained from Myanmar could be divided into four groups (Fig. 1A). A PCA scatter-plot subsequently indicated that these landraces could also be divided into four major groups (Fig. 2A), and the results were consistent with the data obtained by UPGMA clustering. The cumulative contribution rate up to the third component was 74.8%. UPGMA clustering also revealed that the 11 international standard cultivar samples supplied by INIBAP could be distributed to 5 separate groups (Fig. 1B) and this finding was confirmed by PCA scatter-plotting (Fig. 2B). The cumulative contribution rate up to the third component was 64.0% in this case. In addition, almost all the specimens within each of the two origin groups, with the exception of MS11-1, MS11-2, STC15, STC51 and STC52, were clustered together by UPGMA analysis (Fig. 1C), with PCA analysis also showing the same results (Fig. 2C). In this instance, the cumulative contribution rate up to the third component was 54.6%.

These findings indicate that although the genetic diversity profile of the Myanmar banana specimens was different from that of international standard cultivars for a few primer-sets, the average levels of diversity between them were not distinctly different. However, UPGMA and PCA analyses also showed that most specimens within the Myanmar landrace and international standard cultivar groups tended to be clustered/spotted together. Additional marker sets will therefore be needed to obtain more detailed information about these two groups. In addition, some Myanmar landraces and international cultivars, such as MS11-1, MS11-2, STC15, STC51 and STC52, were found to be more highly polymorphic. We further observed that the MS2-1 and MS2-2, MS11-1 and MS11-2 landraces were not genetically identical based on PBA marker information, although they had the same original name (Table 1). Landraces differing in the skin color were included in this study, but we were unable to obtain appropriate molecular markers to analyze them.

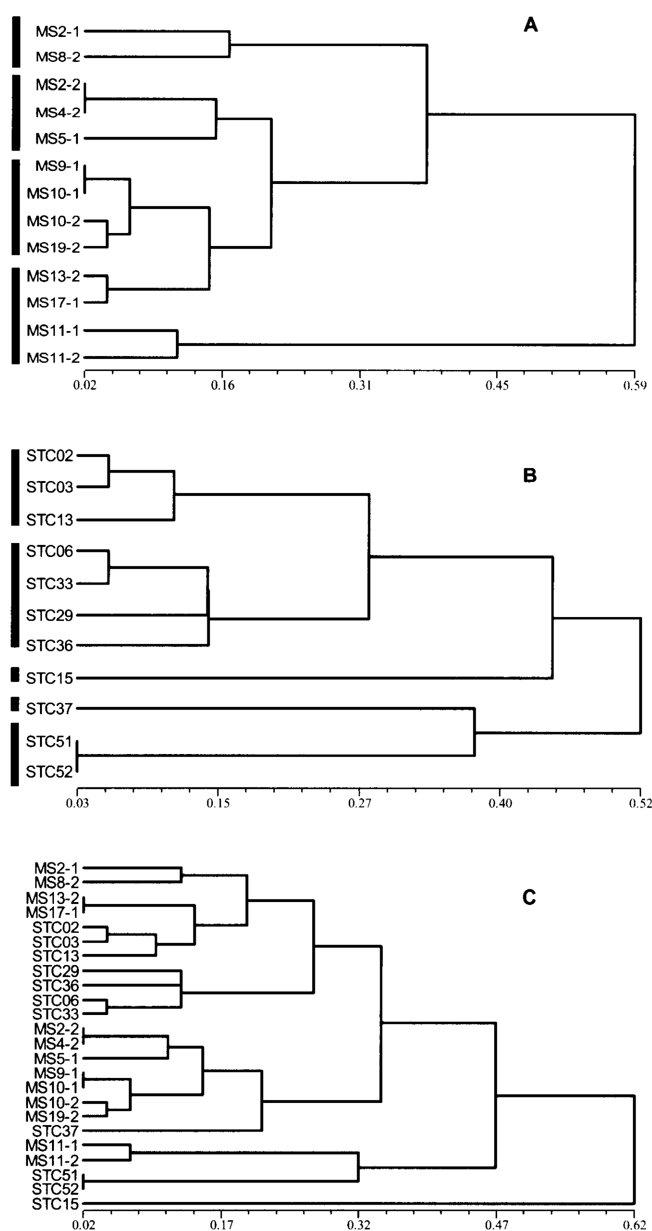

Fig. 1. PBA marker-based dendrograms indicating genetic diversity levels among banana landraces and cultivars. The horizontal axis shows the genetic similarity coefficient (Jaccard 1908). The vertical lines on the left side of the accession names indicates the different groups. A: Myanmar landraces, B: international standard cultivars, C: combined materials.

Genetic diversity is used by breeders worldwide to produce improved varieties of plants. Hence, the genetically diverse resources for the genus *Musa* provide the basic elements that could contribute to improved production of this internationally important crop. Successful breeding efforts

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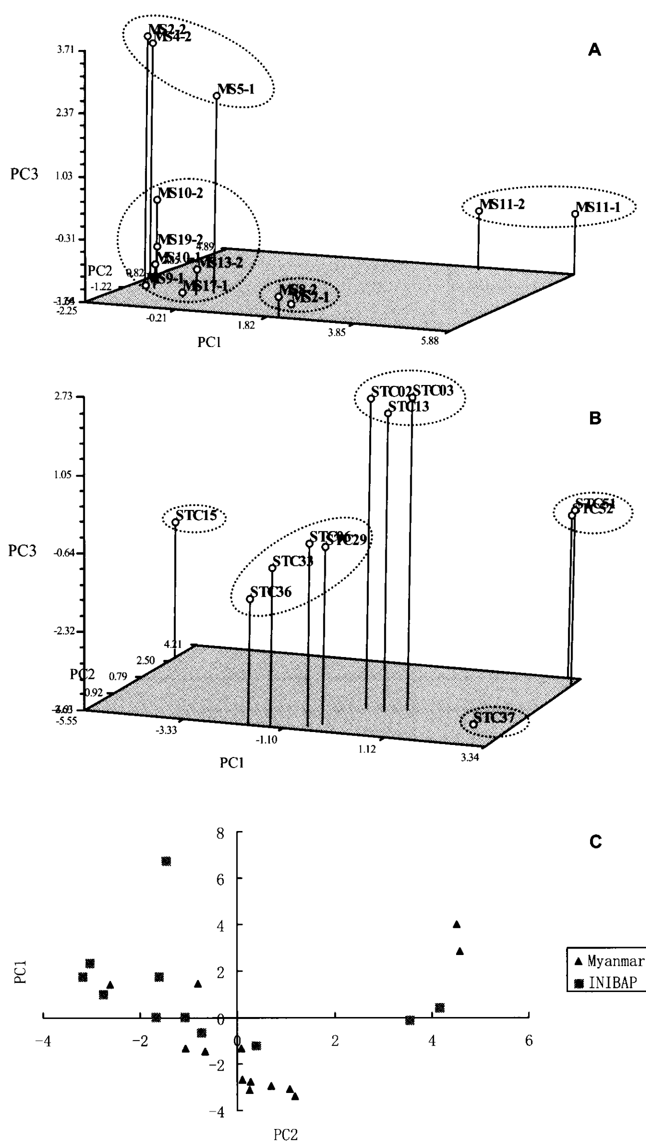

Fig. 2. PCA scatter-plots of banana landraces and cultivars based on PBA markers. PC: principal component. The circles indicate different groups. A: Myanmar landraces; the contribution of the first PC is 43.6%, the contribution of the second PC is 20.3%, the contribution of the third PC is 10.9%. B: international standard cultivars; the contribution of the first PC is 29.1%, the contribution of the second PC is 22.5%, the contribution of the third PC is 12.4%. C: combined materials; the contribution of the first PC is 27.1%, the contribution of the second PC is 17.1%.

thus facilitate the growth of these crops in a wide range of environments that can meet the varied needs of the millions of people who depend on them for both food and income. Breeders who sought genetic materials to initiate projects for the restoration of some of the diversity that had been lost from commercial banana plantations, have used these materials primarily from Asian countries, where the banana is known to originate (INIBAP 2003). Myanmar is such a country and the analysis of polymorphisms in Myanmar landraces is, therefore, useful for promoting banana cultivar improvement. The findings of the present study provide useful information for such future projects.

Acknowledgements

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