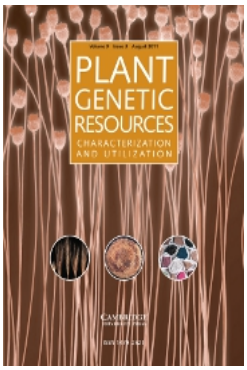

Propagation management methods have altered the genetic variability of two traditional mango varieties in Myanmar, as revealed by SSR

R. Hirano, H. Ishii, Than Htun Oo, S. A. Gilani, A. Kikuchi and K. N. Watanabe

Plant Genetic Resources / Volume 9 / Issue 03 / August 2011, pp 404 - 410
DOI: 10.1017/S1479262111000049, Published online: 01 February 2011

ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2011) **9**(3); 404–410
doi:10.1017/S1479262111000049

Propagation management methods have altered the genetic variability of two traditional mango varieties in Myanmar, as revealed by SSR

R. Hirano[1], H. Ishii[1], Than Htun Oo[2], S. A. Gilani[1], A. Kikuchi[1] and K. N. Watanabe[1]*

[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Tennodai, Tsukuba, Ibaraki 305-8572, Japan and* [2]*Vegetable and Fruits Research and Development Centre, Myanma Agriculture Service, Ministry of Agriculture and Irrigation, Hlegu, Yangon Division, Myanmar*

Received 14 September 2010; Accepted 23 December 2010 – First published online 1 February 2011

Abstract

Mango (*Mangifera indica* L.) is an important fruit crop with a long cultivation history in Myanmar. This study evaluated the genetic variation within two economically important traditional varieties, 'Yin Kwe' and 'Sein Ta Lone', and the relationship between genetic variation and propagation practices. Genetic variation was estimated by genotyping 94 individuals with 12 single sequence repeat markers. 'Yin Kwe' ($n = 53$) showed higher levels of observed heterozygosity ($H_o = 0.59$) and average genetic distance among individuals ($D_a = 0.29$) than did 'Sein Ta Lone' ($n = 41$; $H_o = 0.45$; $D_a = 0.09$). The differences between the two varieties at the DNA level were significant ($F_{st} = 0.44$). The broader genetic background in 'Yin Kwe' compared with 'Sein Ta Lone' was also demonstrated by neighbour-joining and principal coordinates analyses. Differences in variety uses and propagation practices were determined by interviewing local specialists in Lower Myanmar (southern Myanmar). 'Yin Kwe' was often used as a rootstock for 'Sein Ta Lone'. Clonal propagation by grafting was observed frequently for 'Sein Ta Lone' but never for 'Yin Kwe'. The differences in genetic variation between these two varieties might have been caused by the propagation practices for each variety, which result from their respective uses.

Keywords: genetic diversity; landraces; *Mangifera indica*; Myanmar; propagation management; traditional variety

Introduction

Selection by farmers over thousands of years has enriched the genetic pool of many crops, including fruit trees, by promoting intraspecific diversity (Frankel *et al.*, 1995). These kinds of farmer-selected crop varieties are often referred to as traditional varieties or landraces. Camacho Villa *et al.* (2005) defined a landrace as 'a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems'. Applying this definition, traditional mango varieties in Myanmar can be considered as landraces.

Mango (*Mangifera indica* L.) is one of the most important fruit crops in Myanmar because of its agronomic and cultural value. The bibliographic record of mango cultivation in Myanmar can be traced back to the 5th century

* Corresponding author. E-mail: nabechan@gene.tsukuba.ac.jp

AD (Hirano *et al.*, 2008). Although the exact number is not known, more than 200 landraces are recognized in the country (K. Win, pers. commun., 2008). A broad range of diversity has been nurtured through the long history of mango cultivation but so far, no formal breeding has been carried out in Myanmar. Most of the mango production is consumed domestically, but exports have grown in recent years (Kyaw Htu, 2007). The traditional varieties 'Yin Kwe' and 'Sein Ta Lone' (Supplementary Fig. S1, available online only at http://journals.cambridge.org) are two of the most popular varieties in Myanmar. 'Yin Kwe' is a representative traditional variety that is grown throughout the country. Its fruiting period is relatively long, and it bears numerous fruits (Myanma Agriculture Service, 2005). 'Yin Kwe' is also used as a rootstock of other varieties. 'Sein Ta Lone' is one of the most popular varieties because of the aroma and sweetness of its fruits. This variety is traded at higher prices in the domestic market as well as in border trades due to the excellent fruit quality. 'Sein Ta Lone' is distributed in the central (Central Myanmar) and southern (Lower Myanmar) parts of the country.

Single sequence repeat (SSR) markers for mango have been designed by several researchers as a tool to study genetic variation (Duval *et al.*, 2005; Honsho *et al.*, 2005; Schnell *et al.*, 2005; Viruel *et al.*, 2005). The broad genetic background and genetic distinctiveness of mango in Myanmar have been revealed by SSR analyses comparing Myanmar mango varieties with those from other parts of the world (Hirano *et al.*, 2010). A high degree of genetic variability is expected within varieties considered to be landraces, but the genetic variation within most varieties of mango in Myanmar is still unknown.

The method of propagation might be one of the most influential factors affecting heterogeneity and genetic variation within traditional mango varieties in Myanmar. For example, a landrace that is propagated using rooted cuttings or grafts will remain more homogeneous than one that is propagated by self-pollination or by open crosses with other landraces. The level of genetic variation may affect the uniformity of a variety because the genetic variation may change the morphological characteristics. It is, therefore, essential to study the genetic variation within and between varieties and to understand its relationship with traditional propagation practices. This study evaluated the genetic variation within two economically important varieties, 'Yin Kwe' and 'Sein Ta Lone', by using SSR markers. The degree of variation was assessed in light of the traditional propagation practices used for the two varieties. The study results will not only provide an example of the relationship between traditional management and genetic variation but will also provide insights into conservation and sustainable utilization of the species in Myanmar.

Materials and methods

Plant materials

Leaves of 'Yin Kwe' and 'Sein Ta Lone' were sampled in different orchards of the Yangon Division in Myanmar in January and February 2008. One or two young leaves were collected per tree and kept in a plastic bag with silica gel until the leaves were completely dried. The collection consisted of 53 individuals of 'Yin Kwe' and 41 individuals of 'Sein Ta Lone' (Table 1). Traditional propagation practices used for the two varieties were assessed through observation of the seed nursery and interviewing local specialists who are actively involved in mango cultivation and harvesting practices in the Yangon and Bago districts. Orchards, nurseries and local retailers of seedlings were also visited to observe the local propagation methods.

SSR analysis

DNA was extracted from dried leaves of each accession by using the CTAB; Cetyltrimethyl Ammonium Bromide method (Doyle and Doyle, 1987). A total of 24 SSR markers designed to amplify dinucleotide-repeat microsatellite loci were tested (Duval *et al.*, 2005; Schnell *et al.*, 2005; Viruel *et al.*, 2005). PCR conditions and procedures were the same as in Hirano *et al.* (2010). Each reaction mixture contained 1 × Ex Taq Buffer (Takara Bio Inc., Otsu, Shiga, Japan), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 U of Ex Taq polymerase (Takara Bio Inc.) and 10 ng of genomic DNA in a 10 μl total reaction volume. PCR conditions were 95°C for 5 min; followed by 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min; followed by a final extension of 72°C for 5 min. After checking for amplification of target loci by electrophoresis in 1.5% agarose gel, samples were analyzed by capillary electrophoresis with an automated DNA sequencer (ABI model 3130; Applied Biosystems Inc., Carlsbad, CA, USA). The fragment patterns were scored using the Gene Mapper 3.0 software (Applied Biosystems Inc.). The extracted data were exported as allele sizes and formatted for further statistical analysis. Mango is diploid, so to identify markers that detected single loci, any markers that produced more than two alleles (bands)/individual were eliminated. Consequently, we selected 12 SSR loci for further analysis (Table 2).

Data analysis

Genetic parameters were calculated for 94 individuals (41 'Sein Ta Lone' and 53 'Yin Kwe') over the 12 SSR loci. Genotypes showing one and two bands were scored as homozygous and heterozygous, respectively.

Table 1. Collection locations and number of samples

Collection location	Samples collected	
	'Yin Kwe'	'Sein Ta Lone'
Vegetable and Fruit Research and Development Center, Hlegu, Bago Division (17°09′11″ N, 96°17′27″ E)	32 Samples (MM111, MM114, MM257, MM258, MM259, MM260, MM261, MM262, MM263, MM264, MM265, MM266, MM267, MM268, MM269, MM270, MM271, MM272, MM273, MM274, MM275, MM276, MM277, MM278, MM279, MM280, MM281, MM282, MM283, MM284, MM285 and MM286)	Seven samples (MM105, MM107, MM109, MM112, MM113, MM115 and MM116)
Kan Thar Yar Farm, MAS, Taikkyi Township, Yangon Division (17°20′11″ N, 95°59′08″ E)	20 Samples (MM237, MM238, MM239, MM240, MM241, MM242, MM243, MM244, MM245, MM246, MM217, MM218, MM219, MM220, MM221, MM222, MM223, MM224, MM225 and MM226)	32 Samples (MM301, MM302, MM303, MM304, MM305, MM306, MM307, MM308, MM309, MM310, MM311, MM312, MM313, MM314, MM315, MM316, MM317, MM318, MM319, MM320, MM321, MM322, MM323, MM324, MM325, MM326, MM327, MM328, MM329, MM330, MM331 and MM332)
Myanmar Agriculture Service, Bago office, Bago Division (17°20′01″ N, 96°30′43″ E)	One sample (MM188)	Two samples (MM185 and MM189)
Total	53	41

For the purpose of this analysis, the two homozygous classes were grouped together. The genetic variation at each locus was measured in terms of the number of observed alleles. Observed heterozygosity (H_o) and expected heterozygosity (H_e) (Hartl and Clark, 1997) were calculated for each locus. Hardy–Weinberg equilibrium was tested for each locus in both varieties using Genepop version 4.0.7. The polymorphic information content (PIC) value for each marker was calculated using the PowerStats program, version 1.2 (Tereba, 1999).

Table 2. SSR markers used in this study

Locus	GenBank accession no.	Alleles (no.)	Size range[a] (bp)
LMMA4	AY628376	4	242–260
LMMA5	AY628377	5	294–300
LMMA8	AY628380	16	218–289
LMMA9	AY628381	7	186–233
LMMA10	AY628382	8	166–194
LMMA11	AY628383	7	250–264
LMMA12	AY628384	5	214–220
LMMA14	AY628386	10	180–215
LMMA15	AY628387	6	223–237
miSHRS29	AY942822	4	191–202
miSHRS37	AY942828	5	142–148
mMiClR014	AJ635176	8	166–186

[a] Including length of tail sequences (18 bp total).

Nei's genetic distance (Nei *et al.*, 1983) between each individual was measured using Population 1.2.30 (Langella, 1999). Using the same program, a dendrogram was constructed with the neighbour-joining method. Bootstrap permutations were performed 1000 times.

To present a graphical representation of genetic relationships between each accession of the two landraces, principal coordinates analysis was carried out on a matrix of Nei's genetic distances and a scatterplot of the first two principal coordinates.

The *F* statistic, F_{st}, was calculated in an analysis of molecular variance (AMOVA; Peakall *et al.*, 1995) framework, which also allowed for significance testing by random permutation (999 permutations). A codominant allelic distance matrix was used as input into the AMOVA analysis. The F_{st} statistic measures the proportion of the genetic variance between populations relative to the total variance, giving an indication of the degree of population differentiation. The F_{is} and F_{it} measures within-population variation and total-population variation, respectively. Each value was calculated as follows: $F_{st} = AP/(WI + AI + AP)$; $F_{is} = AI/(WI + AI)$; $F_{it} = (AI + AP)/(WI + AI + AP)$, where AP is the estimate variance among population (varieties in this case), AI is the estimate variance among individuals and WI is the estimate variance within individuals (Peakall *et al.*, 1995). All calculations were performed using the program GenAlEx6.1 (Peakall and Smouse, 2006).

Results

Genetic variation revealed by SSR

We assessed the allelic variation of two representative traditional varieties of mango, 'Yin Kwe' and 'Sein Ta Lone', by means of SSR analysis of 85 alleles at 12 loci (Table 2). Forty-seven alleles were detected only in 'Yin Kwe', and six alleles were detected only in 'Sein Ta Lone' (data not shown). The number of effective alleles (N_a)/locus ranged from 2 to 6 (mean 3.25) for 'Sein Ta Lone' and from 4 to 14 (mean 6.50) for 'Yin Kwe', respectively (Table 3). The average H_o was 0.45 and 0.59 for 'Sein Ta Lone' and 'Yin Kwe', respectively. 'Yin Kwe' showed a higher level of genetic diversity, with an average genetic distance (D_a) of 0.29, whereas D_a within 'Sein Ta Lone' was 0.09.

The two varieties were clearly separated in the neighbour-joining tree, except for two individuals of 'Sein Ta Lone' (MM301 and MM302) that were grouped within the 'Yin Kwe' part of the tree (Fig. 1). Principal coordinates 1 and 2 accounted for 68.1 and 12.2% of the total variance, respectively (Fig. 2). The principal coordinates plot demonstrated two major clusters of 'Sein Ta Lone' and 'Yin Kwe' on the PC1 axis, with the exception of 'Sein Ta Lone' individuals MM301 and MM302.

The AMOVA and *F* statistics results showed that most of the genetic variance existed within individuals (39% of total variance and $F_{it} = 0.32$, $P = 0.001$) and among varieties (61% of total variance and significant $F_{st} = 0.44$, $P = 0.001$) (Table 4). The estimated F_{is} (among individuals within a variety) was negative (-0.22) because of the negative estimated value of the variance components among individuals; this negative estimate indicates that the true value of the estimation is zero. A negative value of F_{is} indicates an excess of heterozygotes. Taken together, these results indicated clear genetic differentiation of these two varieties and a different level of genetic diversity within each variety.

Traditional propagation methods and the usage of 'Sein Ta Lone' and 'Yin Kwe' in Lower Myanmar

'Sein Ta Lone' originated in Central Myanmar (near Mandalay), and it is believed to be relatively new to Lower Myanmar. Local specialists and nursery workers reported that most 'Sein Ta Lone' is clonally propagated through grafting. In almost all cases, 'Sein Ta Lone' was grafted onto a stock of 'Yin Kwe'. The scion of 'Sein Ta Lone' was generally chosen from the most productive adult tree within the orchard or nursery. 'Sein Ta Lone' was reported to be a polyembryonic type of mango. Polyembryonic mangoes can produce several nucellar embryos within a single seed; these embryos are genetically identical to the mother plant.

The long cultivation history of 'Yin Kwe' in Myanmar was reported to us by local specialists. 'Yin Kwe' is commonly grown in home gardens, and it is widely distributed across the country. There was no evidence of grafted trees of 'Yin Kwe'. According to the local specialists, 'Yin Kwe' is the best variety for rootstocks in Lower Myanmar due to its adaptation to the local environment. 'Yin Kwe' was also reported to be polyembryonic. Local farmers remove all the seedlings from each seed except the most vigorous one; the seedlings are grown for about 1 year and then used as rootstocks for other varieties.

Table 3. SSR markers used in this study and summary statistics for each variety

	'Sein Ta Lone'						'Yin Kwe'					
Locus	*N*	N_a	H_o	H_e	PIC	Departure from HWE[a]	*N*	N_a	H_o	H_e	PIC	Departure from HWE
LMMA4	41	2	0.05	0.05	0.05	NS	53	4	0.87	0.55	0.44	***
LMMA5	41	2	0.00	0.22	0.19	***	53	5	0.13	0.33	0.32	***
LMMA8	41	5	1.00	0.55	0.44	***	53	14	0.25	0.46	0.44	***
LMMA9	41	3	0.05	0.10	0.09	***	53	6	0.85	0.68	0.62	***
LMMA10	41	4	1.00	0.55	0.44	***	53	8	0.91	0.70	0.64	***
LMMA11	41	2	0.98	0.51	0.38	***	53	6	0.17	0.30	0.29	***
LMMA12	41	3	1.00	0.52	0.39	***	53	5	0.96	0.55	0.44	***
LMMA14	41	5	0.78	0.56	0.46	***	53	10	0.62	0.69	0.64	***
LMMA15	41	3	0.05	0.10	0.09	***	53	6	0.64	0.58	0.54	***
miSHRS29	41	2	0.00	0.09	0.09	***	53	4	0.98	0.60	0.51	***
miSHRS37	41	2	0.37	0.30	0.25	NS	53	5	0.62	0.65	0.57	***
mMiClR014	41	6	0.12	0.31	0.30	***	53	5	0.06	0.13	0.12	***
Mean	41	3.25	0.45	0.32	0.26		53	6.50	0.59	0.52	0.46	
SE		0.41	0.13	0.06				0.84	0.10	0.05		

[a] HWE, Hardy–Weinberg equilibrium.
NS, not significant; ***, Highly significant ($P < 0.001$).

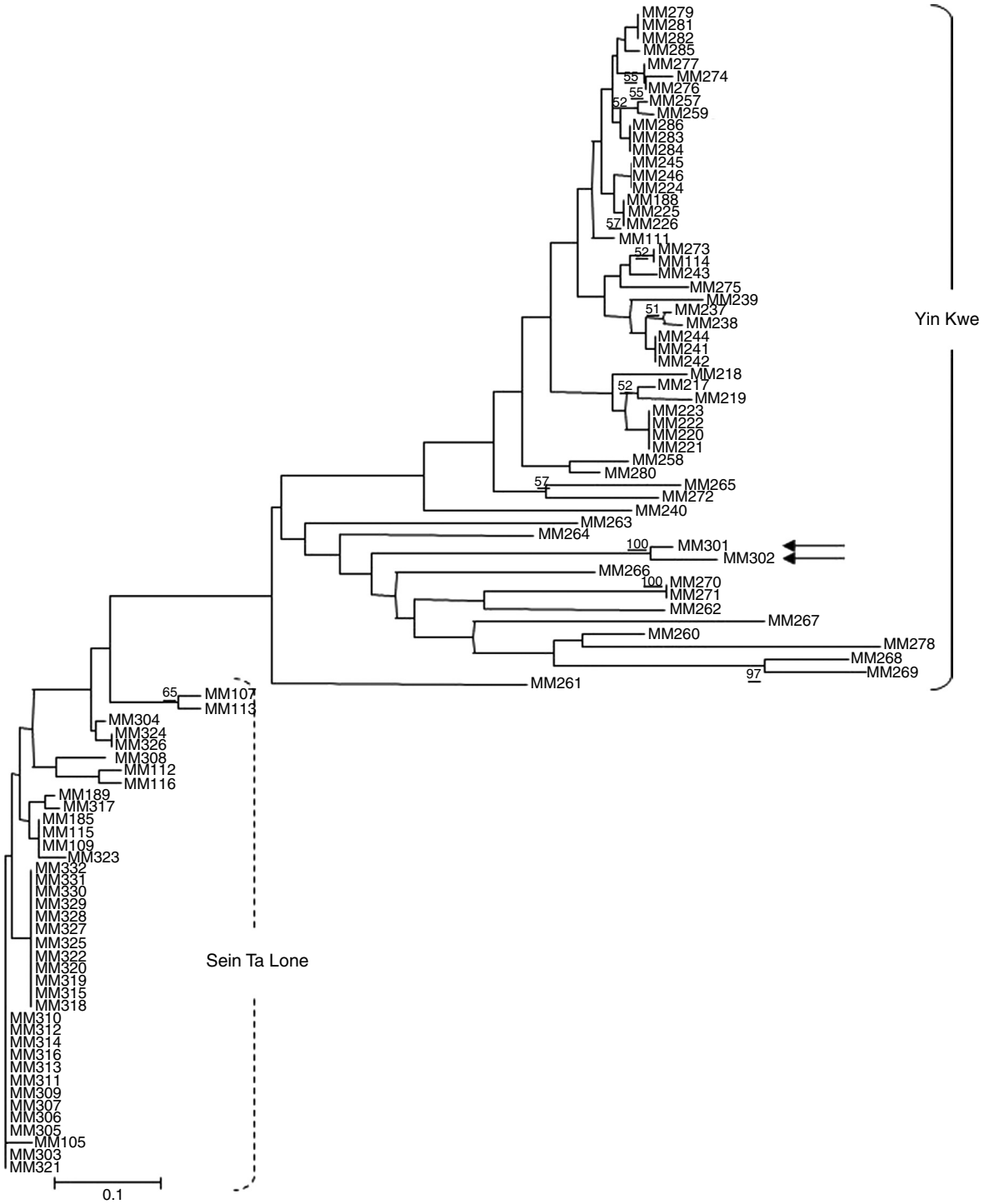

Fig. 1. Neighbour-joining dendrogram of 'Yin Kwe' and 'Sein Ta Lone'. Underlined numbers on branches indicate bootstrap values obtained by using 1000 permutations. Only bootstrap values larger than 50 are indicated. Arrows indicate individuals of 'Sein Ta Lone' that fell within the 'Yin Kwe' portion of the tree.

Discussion

In this study, significant levels of genetic variation were observed within the traditional varieties 'Sein Ta Lone' and 'Yin Kwe'. Higher levels of genetic variation and heterogeneity were observed in 'Yin Kwe' than in 'Sein Ta Lone' in terms of number of alleles per locus, genetic diversity indices and genetic distances among individuals. The propagation practices were considerably different between these two traditional varieties. The different

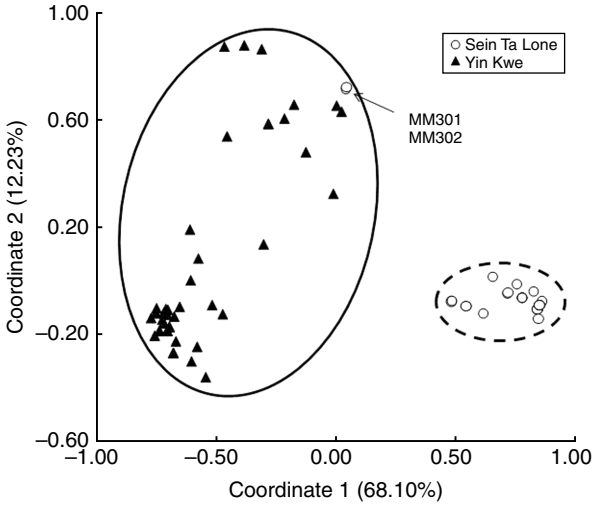

Fig. 2. Principal coordinates plot of 'Sein Ta Lone' and 'Yin Kwe'. Filled triangles indicate 'Yin Kwe', and open circles indicate 'Sein Ta Lone'. Arrows indicate the same individuals as in Fig. 1.

uses of the two varieties were an important factor to explain the differences in propagation modes and degree of genetic variation. 'Sein Ta Lone' was propagated mainly by grafting to maintain its genetic integrity, because the fruit quality and uniformity are important for both producers and consumers. Sets of individuals with zero genetic distances from one another were frequently observed in 'Sein Ta Lone'; these individuals were assumed to be ramets of the same genet. In contrast, no evidence of grafting was found for 'Yin Kwe'. Since the variety is well adapted to Lower Myanmar, it was often used as a rootstock without regard to the degree of uniformity. 'Yin Kwe' contained a high level of genetic variation even though it is polyembryonic. In other studies, genetic variation within a commercial variety and within selections of polyembryonic mango has also been reported (Schnell and Knight, 1991; Bally *et al.*, 1996). Schnell and Knight (1991) reported 0–64% of zygotic off types in different varieties of polyembryonic mango and showed different levels of genetic variation in polyembryonic seedling populations.

The other possible reason for the differences in genetic variation is the history of each traditional variety. 'Sein Ta Lone' was recently introduced into Lower Myanmar, whereas 'Yin Kwe' has a long history of cultivation in the region. In the case of 'Sein Ta Lone", the number of introduced genotypes might have been limited, leading to the low level of genetic variation (e.g. a founder effect). The average H_o of 'Yin Kwe' was equivalent to the heterozygosity observed at the country or regional level for cultivated varieties (Schnell *et al.*, 2006) and Mexican landraces (Gálvez-López *et al.*, 2009) of mango. 'Yin Kwe' was frequently used as a rootstock in Lower Myanmar because it is well adapted to the local environment. The broad genetic variation and adaptation to the local environment supported the long history of cultivation of 'Yin Kwe' in Lower Myanmar.

Clear genetic differentiation between 'Yin Kwe' and 'Sein Ta Lone' was demonstrated by both F_{st} and AMOVA (Table 4). The value of F_{st} was much higher than the threshold for significant population differentiation ($F_{st} = 0.25$; Hartl and Clark, 1997). Mango is considered to be an insect-pollinated tree species that favours cross-pollination (Davenport and Núñes-Elisea, 1997), which might result in less differentiation. Both 'Yin Kwe' and 'Sein Ta Lone' are polyembryonic varieties. The outcross rates of these varieties are not known, but a high amount of clonal regeneration by nucellar embryos (apomixis) might have maintained their genetic integrity and the high F_{st} value between these varieties.

Although almost all the 'Sein Ta Lone' accessions were grouped closely together in the principal coordinates plot, two individuals (MM301 and MM302) were closer to some of the 'Yin Kwe' individuals (Fig. 2). In the dendrogram, these two individuals were grouped together with 'Yin Kwe' (Fig. 1). There are several possible reasons for these results. First, there might have been misidentification of the variety at the orchard. We collected the leaf samples when the fruits, the most prominent key for variety identification, were not available. Second, the sampled leaves might have arisen from the rootstock rather than from the scion. Finally, there might be a greater degree of variation within 'Sein Ta Lone' than was previously believed. These two individuals possessed unique alleles not found either in the other 'Sein Ta Lone' or 'Yin Kwe' accessions. Large-scale genetic analysis of 'Sein Ta Lone', including samples from the

Table 4. AMOVA and *F* values for the mango varieties 'Sein Ta Lone' and 'Yin Kwe'

Source	d.f.	SS	Variance components	*F*	*P*
Among varieties	1	192.460	2.06	$F_{st} = 0.44$	0.001
Among individuals	92	184.641	−0.58	$F_{is} = -0.22$	1.000
Within individuals	94	297.500	3.16	$F_{it} = 0.32$	0.001
Total	187	674.601	5.22		

d.f., degrees of freedom; SS, sum of squares.

place of origin, might reveal greater genetic diversity within this variety.

No large-scale mango breeding programmes have been carried out in Myanmar. The characteristics of traditional varieties of mango have been created by repeated natural selection, formation of zygotic embryos by selfing or hybridization, and mutation. Local farmers have selected and maintained desirable genotypes during the long cultivation history of mango. Although both 'Yin Kwe' and 'Sein Ta Lone' are considered to be landraces, the differences in propagation methods related to the use and history of each variety might have greatly affected their levels of genetic variation. The information on genotypes and genetic variation of these traditional mango varieties obtained in this study can be used as background information for their future variety registration and to develop a strategy for their *in situ* and *ex situ* conservation.

Acknowledgements

We thank Myanmar Agriculture Service/Department of Agricultural Planning for facilitating our collection expedition and interviews in Myanmar. We extend our appreciation to Dr. K. Intabon and Ms. M. Okubo for helping to collect samples during collection expedition in Myanmar. This research was funded by Grant-in-Aid for Scientific Research no. 21405017.

References

Bally ISE, Graham GC and Henry RJ (1996) Genetic diversity of Kensington mango in Australia. *Australian Journal of Experimental Agriculture* 36: 243–247.

Camacho Villa TC, Maxted N, Scholten M and Ford-Lloyd B (2005) Defining and identifying crop landraces. *Plant Genetic Resources* 3: 373–384.

Davenport TL and Núñes-Elisea R (1997) Reproductive physiology. In: Litz RE (ed.) *The Mango: Botany, Production and Uses*. Cambridge: Cambridge University Press, pp. 69–146.

Doyle JJ and Doyle JLA (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Duval MF, Bunel J, Sitbon C and Risterucci AM (2005) Development of microsatellite markers for mango (*Mangifera indica* L.). *Molecular Ecology Notes* 5: 824–826.

Frankel OH, Brown AHD and Burdon JJ (1995) *The Conservation of Plant Biodiversity*. Cambridge: Cambridge University Press. http://www.cup.com.ac.uk/aus/catalogue/catalogue.asp?isbn=9780521467315

Gálvez-López D, Hernández-Delgado S, González-Paz M, Becerra-Leor EN, Salvador-Figueroa M and Mayek-Pérez N (2009) Genetic analysis of mango landraces from Mexico based on molecular markers. *Plant Genetic Resources: Characterization and Utilization* 7: 244–251.

Hartl DL and Clark AG (1997) *Principles of Population Genetics*, 3rd edn. Sunderland, MA: Sinauer Associates.

Hirano R, Khain Pann Witt Hmon, Kawase M and Watanabe KN (2008) Mango *Mangifera indica* L. in Myanmar: a preview. Plant Genetic Resources Monograph PGRM. Series 5. Mimatsu Corporation, Tokyo.

Hirano R, Than Htun Oo and Watanabe KN (2010) Myanmar mango landraces reveal genetic uniqueness over common cultivars from Florida, India and Southeast Asia. *Genome* 53: 321–330.

Honsho C, Nishiyama K, Eiadthong W and Yonemori K (2005) Isolation and characterization of new microsatellite markers in mango (*Mangifera indica*). *Molecular Ecology Notes* 5: 152–154.

Kyaw Htu (2007) Growing Mango for Commercial Purposes. Myanmar Agricultural Service, Ministry of Agriculture and Irrigation, Myanmar (written in Myanmar language).

Langella O (1999) Populations 1.2.30. Available at http://www.bioinformatics.org/project/?group_id=84 (accessed 12 October 2009).

Myanma Agriculture Service (2005) Mango cultivation, Ministry of Agriculture and Irrigation, Myanmar (written in Myanmar language).

Nei M, Tajima F and Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution* 19: 153–170.

Peakall R and Smouse PE (2006) GENALEX 6: genetic analysis in Excel, population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

Peakall R, Smouse PE and Huff DR (1995) Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss (*Buchloë dactyloides* (Nutt.) Engelm.). *Molecular Ecology* 4: 135–147.

Schnell RJ and Knight RJ (1991) Are polyembryonic mangoes dependable sources of nucellar seedlings for rootstocks? *Proceedings of the Florida State Horticultural Society* 104: 44–47.

Schnell RJ, Olano CT, Quintanilla WE and Meerow AW (2005) Isolation and characterization of 15 microsatellite loci from mango (*Mangifera indica* L.) and cross-species amplification in closely related taxa. *Molecular Ecology Notes* 5: 625–627.

Schnell RJ, Brown JS, Olano CT, Meerow AW, Campbell RJ and Kuhn DN (2006) Mango genetic diversity analysis and pedigree inferences for Florida cultivars using microsatellite markers. *Journal of the American Society for Horticultural Science* 131: 214–224.

Tereba A (1999) Tools for analysis of population statistics. *Profiles in DNA* 2: 14–16.

Viruel MA, Escribano P, Barbieri M, Ferri M and Hormaza JI (2005) Fingerprinting, embryo type and geographic differentiation in mango *Mangifera indica* L. Anacardiaceae. with microsatellites. *Molecular Breeding* 15: 383–393.