Research article

Genetic Identification of Myanmar Sein Ta Lone Mango (*Mangifera indica* L.) Landrace from the Different Eco-Geographic Regions using Microsatellite Markers

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Abstract Eighty eight Sein Ta Lone mango individuals from five Sein Ta Lone orchards (populations), namely, Mya Na De, Htone Bo, Se Bauk, Taik Kyi, and Nam Latt, and 100 years old plant as control, and a landrace of Yin Kwe individual, a total of 89 individuals, were genotyped using 38 polymorphic markers showed 147 alleles with an average of 3.87 alleles per locus. Polymorphic information content varied from 0.02 to 0.83 and averaged of 0.37.Twenty four loci were Sein Ta Lone unique markers. Cluster analysis depicted those SP4 plant individuals from Se Bauk stand as an out-group from the rest of Sein Ta Lone individuals which scattered independently from their locations. Principal coordinate analysis confirmed grouping of Sein Ta Lone individuals closely to control Sein Ta Lone (100 years old) rather than Yin Kwe variety, and the first three principal coordinates explained 62.05% of total variation. Analysis of molecular variance explained 75% of genetic variation occurred within populations and 25% among populations. The fixation index (Fst) 0.63, pointed the high level of genetic differentiation among populations, it might be due to Yin Kwe control rather than five Sein Ta Lone populations. Limited gene flow (Nm = 0.31) occurred among the populations. Almost the same value (0.32 to 0.35) of the expected heterozygosity and the observed heterozgosity (0.16) were observed in Sein Ta Lone populations. The number of effective alleles varied from 1.88 to 2.13, Shannon Index ranged from 0.54 to 0.61, and same positive fixation index of (0.4), same value of the percentage of polymorphic loci (52.63%). Pairwise Nei genetic identity showed higher value to Sein Ta Lone control (100 years old), highest among five populations ranged from 0.916 to 0.957.

Keywords genetic identification, Sein Ta Lone populations, Sein Ta Lone unique marker, cluster analysis, analysis of molecular variance

INTRODUCTION

Mango (*Mangifera indica)* consisted of two distinct types such as monoembryonic and polyembryonic type. Based upon taxonomic and molecular evidence, the mango (polyembryonic type) probably evolved in north-western Myanmar, Bangladesh and north-eastern India (Bompard, 1993; Mukherjee and Litz, 2009). Hirano et al. (2010) identified the uniqueness of Myanmar mango individuals from Vegetable and Fruits Research and Development Center (VFRDC), Yangon, using 11 SSR markers and results revealed that mango individuals from Myanmar were distinguishable from those of Florida, India, and Southeast Asia.

Polyembryonic mango seeds contain several nucellar and zygotic embryo. Since adventitious embryos develop from the nucellus, or can also originate by direct budding from the cotyledons and hypocotyls of other nucellar, embryos seedlings are genetically identical to the maternal parent (Juiano, 1934). However, there was a little variation among seedlings derived from polyembryonic mangoes, due to somatic mutation (Mukherjee and Litz, 2009) and various biological and cultural factors (Begum et al., 2013). Even, mango has cross-pollinated nature; Moore and Castle (1988) recorded about 88-94% of self-pollinated zygotic seedlings using isozymes.

Nowadays, orchards are being used grafted plants (stock and scion), however, home yard gardens are still using plants directly from seeds. Since Sein Ta Lone mango previously sown from seed, and to date grafted Sein Ta Lone orchards were necessary to document their DNA profile for fingerprinting and genetic variability on farm.

OBJECTIVE

This study aims to analyze genetic diversity and population structure of Sein Ta Lone individuals, using SSR markers.

METHODOLOGY

From May to July, 2017, the leaf samples from 88 Sein Ta Lone sample plants: 18, 15, 18, 18 and 18 sample plants from Mya Na De, Htone Bo, Se Bauk, Taik Kyi, and Nam Latt, respectively, and one control Sein Ta Lone (assuming 100 years old according to villagers of Pae Gin Village, Kyauk Se Township, Mandalay region), were collected. Mya Na De, Htone Bo and Se Bauk orchards are from Mandalay region, Taik Kyi orchard from Yangon region, and Nam Latt orchard from Shan region, respectively. In addition, Yin Kwe landrace, which widely used as rootstock for Sein Ta Lone orchards, was also used as control. This experiment was carried out at the Plant Biotechnology Center (PBC), Department of Agriculture (DOA), Myanmar.

Total genomic DNA was isolated from pale-brown young leaves using the hexadecyl trimethyl ammonium-bromide (CTAB) method (Doyle and Doyle, 1987) with few modifications. A total volume of 10 μl of reaction mixture of PCR cocktails were performed in Labnet (Labnet international Inc.) thermal cycler programmed for 5min at 94°C for initial Denaturation, 35 cycles of 1 min at 94°C, min at 55°C - 60°C (marker dependent), 1 min at 72°C and final extension at 72°C for 5 minutes. PCR products were fractionated through 8% (w/v) polyacrylamide gel and the expressions of gels were recorded on UV light using Cannon G12 digital camera.

SSR fragment sizing was performed with LabImage 2.7.1 Software and genetic distance and cluster analyses were evaluated for 89 individuals with 38 polymorphic loci using NTSYS-pc 2.01 program (Rohlf, 2000). Nei and Li's genetic distance (1979) was conducted to calculate pair-wise genetic distance among all individuals and the dendrogram was constructed by using a distance matrix using the unweighed pair group method with arithmetic average. The polymorphic information content (PIC) was calculated by applying the formula provided by Anderson *et al.*

(1993). To elucidate the genetic relationship among the populations, principal coordinate analysis was conducted using Genalex6.2 (Peakall and Smouse, 2006).The F_{st} value was, in turn, used to estimate gene flow (N_m) as $N_m = (1/F_{st}-1)/4$ (Whitlock and McCauley, 1999).The number of alleles per locus (N_a), the effective number of alleles per locus (N_e), and the genetic heterozygosity (H) were used to detect the level of population genetic diversity.

RESULTS AND DISCUSSION

Genetic Diversity Analysis

The 89 mango individuals from five orchards (viz. Mya Na De, Htone Bo, Se Bauk, Taik Kyi, Nam Latt), were assessed by using 64 SSR markers. A total of 58 markers were well amplified; 20 were monomorphic and 38 were polymorphic markers (Table 1). Among the polymorphic markers, 24 were Sein Ta Lone unique markers; 18 homozygous loci (MiIIHR03a, MiIIHR05c, MiIIHR07a, MiIIHR12a, MiIIHR16a, MiIIHR22a, MiIIHR25a, MiIIHR29a, MiIIHR31b, MiIIHR32a, MiIIHR33a, MiIIHR34b, SSR-16, SSR-19, SSR-20, SSR-52, SSR-89 and MiMRD_l656), and 6 heterozyous loci (MiIIHR02c, MiIIHR04c, MiIIHR17b, MiKVR_a009, MiKVR_ao28 and MiIIHR_f879).

A total of 147 alleles were detected, the average number of alleles per locus was 3.87, with a range from 2 to 8 (Table 1). Polymorphic Information Content (PIC) values ranged from 0.02 to 0.83 with 0.37 averaged, showing that a moderately low diversity exists in mango individuals under study.

Cluster Analysis

A total of 89 mango individuals using 38 SSR were subjected into cluster analysis based on UPGMA. In group I, Sein Ta Lone control (100 years old) clustered together with 12 Sein Ta Lone individuals (viz. NL17, TK1, TK15, MND1, MND20, MND8, MND2, SP14, SP15, HB18, MND16 and TK2) with genetic similarity of (0.86, 0.83, 0.8, 0.79, 0.83, 0.77, 0.75, 0.85, 0.75, 0.75, 0.8, and 0.76, respectively) (Fig. 1). Group II comprised of most of the individuals from five orchards were clustered independent of their locations. Group III consisted of the ten individuals (TK3, TK5, TK6, TK4, TK14, TK13, TK7, TK12, TK18, and TK20), from Taik Kyi orchard were clustered together. Group IV depicted that Yin Kwe stand as separately from other. Interestingly, Se Bauk (SP4) had distinct away the rest of Sein Ta Lone.

In dendrogram, individuals from Mya Na De orchard, MND1 and MND20 showed 0.89 and MND10 and MND13 showed 0.86 genetic similarity and cluster together. In Htone Bo orchard, HB6 and HB15 had 0.91, HB9 and HB10 had 0.88, HB5 and HB13 had 0.84, and HB1 and HB8 had 0.82, genetic similarity and cluster together. For Se Bauk orchard, SP10 and SP13 had 0.93, both SP2 and SP3, and SP1 and SP5 had 0.86, genetic similarity, and clustered as a pair. Similarly, individuals from Taik Kyi orchard, both pairs of TK1 and TK15, TK4 and TK14 had 0.90, TK18 and TK20 had 0.88, TK7 and TK12 had 0.87, TK3 and TK5 had 0.86, and TK10 and TK16 had 0.85, genetic similarity and clustered together. Likewise, individuals from Nam Latt orchard, NL9 and NL10 had 0.90, NL3 and NL6 had 0.86, NL7 and NL8 had 0.84, NL15 and NL16 had 0.78 and NL18 and NL20 had 0.77, genetic similarity, and clustered together.

Principal Coordinate Analysis

In order to explore the distribution pattern of genetic diversity contained in individuals of each population, a principal coordinate analysis was performed on 38 markers in 89 mango individuals (including Sein Ta Lone-100 years old and Yin Kwe controls). DNA scored of these two controls was arbitrarily doubled in order to assume as separate populations. Therefore, a total of 91 individuals were subjected into multivariate analysis. Similar as cluster analysis, PCoA clearly depicted all the individuals from five Sein Ta Lone orchards showed closely grouped to control

Sein Ta Lone (pop2) rather than Yin Kwe (pop1) (Fig. 2).The first three PC showed 62.05 % of total variations, PC1 explained 29.75% of total variations, and PC2 and PC3 showed 17.90% and 14.39%,respectively. Sein Ta Lone controls (100 years old sample, pop2) were close to most of Sein Ta Lone individuals from Nam Latt orchard (Shan region), Se Bauk orchard (Mandalay region), Mya Na De orchard (Mandalay region).

Table 1 Polymorphic markers with their product size, motif, no. of alleles amplified and PIC value

No.	Marker name	SSR motif	No. of alleles	Size (bp)	PIC
1	MiIIHR09c	(CT)3TTGC(CT)2GT(CT)4TC(GT)2(CT)2	7	284-300	0.79
2	MiIIHR21b	(GTTT)3(GT)2TTTTGTC(TG)4(AATGA)2	8	258-275	0.76
3	MiIIHR24b	(CA)9TACC(CATA)6	6	242-252	0.78
4	MiKVR_a394	(TG)7	5	245-256	0.78
5	MiKVR_d864	(ATC)4	7	282-300	0.78
6	MiMRD_l744	(AAAT)4	7	283-303	0.8
7	MiKVR_a152	(ATTAT)4	5	253-265	0.73
8	MiKVR_a965	(AAAAT)4	7	278-300	0.82
9	MiKVR_c273	(TAAAA)5	6	239-255	0.8
10	MiKVR_d656	(TAAAA)4	7	252-277	0.81
11	MiKVR_n259	(CACCCA)4	6	248-266	0.71
12	MiKVR_n613	(TGATGG)4	7	244-264	0.83
13	MiKVR_u796	(GGAAGG)5	4	208-216	0.67
14	MiKVR_t130	(GAAAAA)4	5	255-267	0.72
15	MiIIHR02c	(CA)2A(CA)7AG(CA)5	3	176-239	0.51
16	MiIIHR04c	(CA)11	4	170-202	0.51
17	MiIIHR03a	(CTT)6(CA)2	2	237-243	0.02
18	MiIIHR05c	(CT)8C(CT)2TTTT(CT)4	2	207-213	0.02
19	MiIIHR07a	(GA)11	2	170-192	0.02
20	MiIIHR12a	(GA)11	2	174-180	0.02
21	MiIIHR16a	(GA)10	2	205-211	0.02
22	MiIIHR17b	(GT)13GAGT(GA)10	4	248-284	0.51
23	MiIIHR22a	(GTCTC)2(TGTCTC)3T(CTC)2	2	224-230	0.02
24	MiIIHR25a	(GTTT)3ATTTG(ATT)2	2	147-156	0.02
25	MiIIHR29a	(GT)10	2	153-158	0.02
26	MiIIHR31b	(GAC)6	2	213-217	0.02
27	MiIIHR32a	(GA)12	2	194-206	0.02
28	MiIIHR33a	(GA)12	2	171-173	0.02
29	MiIIHR34b	(GGT)9(GAT)5	2	232-240	0.02
30	SSR-16	(TA)2(CA)10TA(CA)3TA(CA)4	2	180-188	0.02
31	SSR-19	(ACACACAT)3(ACACACACAT)3	2	170-254	0.02
32	SSR-20	(AT)14(GT)18	2	248-252	0.02
33	SSR-52	(GA)16	2	113-115	0.02
34	SSR-89	NA	3	105-119	0.04
35	MiKVR_a009	(GT)8	4	200-246	0.51
36	MiKVR_ao28	(TA)	4	221-259	0.51
37	MiMRD_l656	(ATTT)5	3	278-324	0.04
38	MiIIHR_f879	(TTGGAC)4	3	116-131	0.51
		Average	3.87		0.37

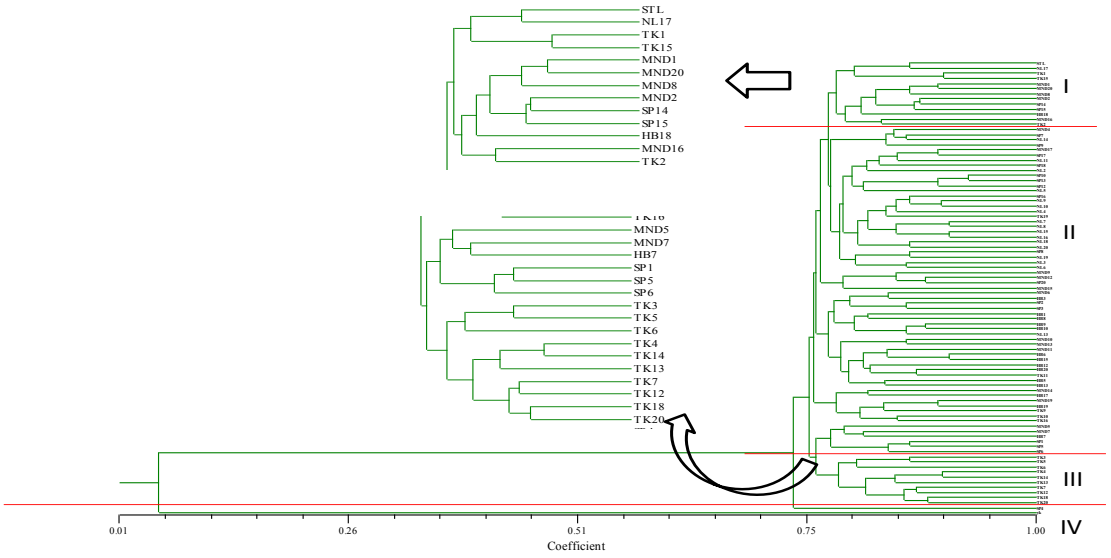

Fig. 1 Dendrogram of 89 mango individuals on the basis of 38 SSR markers using Nei and Li's genetic distance (1979)

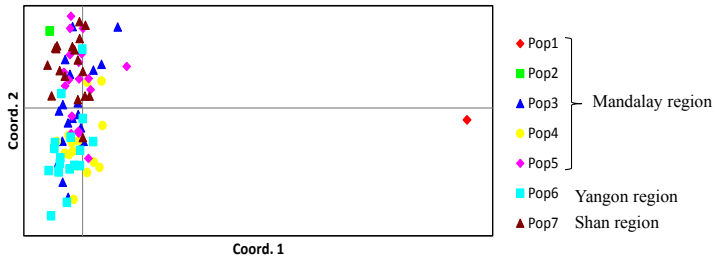

Fig. 2 Two dimensional axis of Principal coordinate analysis (axis 1 vs axis 2) of 91 mango individuals, using 38 markers

pop1-Yin Kwe , pop2 - Sein Ta Lone, pop3 - Mya Na De orchard, pop4 - Htone Bo orchard, pop5- Se Bauk, pop6 - Taik Kyi orchard, and pop7 - Nam Latt orchard

Population Structure and Gene Flow

To determine the pattern of diversity between and within the mango orchards (pop), an analysis molecular variance (AMOVA) in the 91 mango individuals was conducted and results reveal 7 main groups as the orchards which obtained DNA samples and two controls (Yin Kwe and 100 years old Sein Ta Lone). AMOVA using 7 populations suggested that the largest proportion of genetic diversity (75%) was found among plants belonging to the same population, whereas variation present among populations was 25% and no divergence observed between regions, all sources variation were significant at P>0.01 (Table 2). Large variability detected within populations could be explained by the occurrence of gene flow or most probably, by sowing the same materials in different regions. The fixation index (F_{st}), showed the value of 0.63, confirming the high level of genetic differentiation among the populations (Hartl and Clark, 1997). This differentiation might be mainly due to Yin Kwe individuals differ from Sein Ta Lone individuals.

Hirano et al. (2011) mentioned that Yin Kwe and Sein Ta Lone showed significantly variance at DNA level (F_{st}= 0.44). Gene flow (N_m) was equal to 0.31 indicating that limited gene flow among the populations (a local differentiation of populations). It may be due to the occurrence of zygotic seedling (2% to 90%) in polyembryonic cultivars or various biological and cultural practices like continuous grafting from one population to others (Begum et al., 2013). Diaz-Matallana et al. (2009) observed high gene flow value in analysis of diversity among six

Colombian mango populations, and they concluded that it is probably caused by both insect pollinators and human intervention. Since pop1 and pop2, arbitrary population, used the same DNA samples as controls in each pop, there had no consideration for population analysis. Expected heterozygosity (H_e) values of populations from five orchards (i.e. pop3 to pop7) showed 0.31 to 0.34, which pointed that not much difference in population diversity (Table 3). Observed heterozygosity (H_o) was the same value (0.16) in all populations. Observed heterozygosity of each pop was smaller than the expected heterozygosity based on Hardy-Weinberg expectations. Consequently the fixation index was positive over five pops (0.4). The number of effective alleles (N_e) ranged from 1.88 (pop7) to 2.13 (pop 5).The Shannon index (I) ranged from (0.54) in pop7 to (0.61) in pop3 and pop5. The percentage of polymorphic loci (P) across populations was the same value (52.63) %.

Table 2 Analysis of molecular variance for mango individuals

Source	df	SS	MS	Est. Var.	%
Among Regions	2	140.67	70.33	0	0%
Among Pops	4	348.56	87.14	6.84	25%
Within Pops	84	1719.1	20.47	20.47	75%
Total	90	2208.3		27.31	100%

Table 3 Parameters (Mean and SE) of genetic variability of mango populations

Pop*	**N_a**	**N_e**	**I**	**H_o**	**H_e**	**F**	**%P**
Pop 1	1.16±0.06	1.16±0.06	0.11±0.04	0.16±0.06	0.08±0.03	-1±0.00	15.79
Pop2	1.16±0.06	1.16±0.06	0.11±0.04	0.16±0.06	0.08±0.03	-1±0.00	15.79
Pop3	2.55±0.31	2.13±0.22	0.61±0.11	0.16±0.06	0.34±0.06	0.4±0.15	52.63
Pop4	2.37±0.26	1.88±0.16	0.56±0.1	0.16±0.06	0.32±0.05	0.4±0.15	52.63
Pop5	2.47±0.28	2.10±0.21	0.61±0.10	0.16±0.06	0.34±0.05	0.4±0.15	52.63
Pop6	2.42±0.29	1.98±0.20	0.57±0.10	0.16±0.06	0.32±0.05	0.4±0.15	52.63
Pop7	2.26±0.25	1.92±0.18	0.54±0.09	0.16±0.06	0.31±0.05	0.4±0.15	52.63
Total	2.06±0.10	1.76±0.07	0.44±0.04	0.16±0.02	0.26±0.02	0.25±0.06	42.11±6.79

**For abbreviation see Figure 2*

N_a = no. of alleles

N_e = no. of effective alleles = 1/(sum pi^2)

*I = Information index = -1*sum (pi*ln(pi))*

H_o = Observed Heterozygosity =no. of Hets/N

H_e = Expected Heterozygosity = 1- Sum pi^2

F = Fixation Index= $(H_e - H_o) / H_e = 1 - (H_o / H_e)$

%P= Percentage of polymorphic loci

All populations (pop3 to pop7) from five orchards showed high pairwise genetic identity to control Sein Ta Lone (100 years old) (pop2) than Yin Kwe (pop1) (Table 4). Likewise, pairwise genetic identity among five orchards were high, indicating quite similar mango clone, pop7 (Nam Latt orchard) and pop3 (Mya Na De orchard) showed high value of genetic identity (0.831 and 0.822). Pop4 (Htone Bo orchard) showed the lowest value of 0.766. Pop 5 (Se Bauk orchard) showed high genetic identity (0.957) to pop7 (Nam Latt orchard), and (0.951) to pop3 (Mya Na De orchard). Five populations (grafted orchards) showed more similar to each other than Sein Ta Lone (100 years old control), it might be mainly due to cross pollinated nature of mango (gene flow).

Table 4 Pairwise population matrix of Nei genetic identity

Pop*	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7
Pop1	1.000						
Pop2	0.000	1.000					
Pop3	0.084	0.822	1.000				
Pop4	0.096	0.766	0.935	1.000			
Pop5	0.095	0.803	0.951	0.945	1.000		
Pop6	0.055	0.789	0.923	0.929	0.918	1.000	
Pop7	0.072	0.831	0.916	0.917	0.957	0.919	1.000

CONCLUSION

As Sein Ta Lone mango is an economically important crop in Myanmar, this study provides insight into extent and genetic variability at DNA level of five Sein Ta Lone orchards to control Sein Ta Lone (100 years old plant). Genotyping with 38 SSR markers confirmed differentiation of Yin Kwe and Sein Ta Lone at DNA level which was consistent with Hirano et al. (2011). Moderately low genetic diversity in PIC value indicated five Sein Ta Lone orchards(grafted trees) have high genetic similarity to 100 years old Sein Ta Lone control (seeded tree). Moreover, variation within populations (within orchards) were observed and next grafting procedure from present Sein Ta Lone individuals should be carefully carried out for ensure to get true to type Sein Ta Lone mango. Sein Ta Lone unique markers will be useful in varietal identification in mango varieties. This study will provide valuable information to varietal identification and protection, and further varietal improvement program at the national and global perspective.

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