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Genetic Diversity and Genetic Uniqueness of Indigenous Myanmar Mango (Sein Ta Lone) Cultivar in Kyaukse District

Honey Thet Paing Htway, April Nwet Yee Soe, Moe Moe Myint, Khin Pyone Yi, Nay Nwe Nyein Chan, May Sandar Kyaing, Nwe Nwe Soe Hlaing, Su Myat Aung, Seinn Sandar May Phyo, Yin Min Htwe, Chaw Ei Htwe Maung, and San San Yu*

Microbiology Laboratory, Department of Biotechnology Research, Kyaukse, Myanmar

*Corresponding author
San San Yu, Microbiology Laboratory, Department of Biotechnology Research, Kyaukse, Myanmar, Tel: 95-924-01-379; Email: sakurasan2007@gmail.com

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Abstract

Genetic diversity of 60 Myanmar Sein Ta Lone mango accessions from 21 orchards within three locations in Kyaukse District was studied in this research. 9 Simple Sequence Repeat (SSR) markers were used to study the genetic diversity and phylogenetic relationships among the collected mango accessions. Total of 48 scorable bands were observed on amplification with the sizes ranging between 110bp and 369bp. Polymorphic information content (PIC) of 9 SSR markers were 0.265 to 0.74 with an average of 0.421 per marker. The optimal annealing temperature of the primers was 42 to 58°C in range. By using UPGMA cluster analysis, it grouped all the accessions from three locations with a genetic similar coefficient between 0.68-0.96. There was no clone in each orchard and the least dissimilarity was ~ 4% in SM orchard in location 1, SH orchard and SPu orchard in location 2 and STM orchard in location 3. In the present study, analysis of genetic study by means of microsatellite markers showed high genetic diversity of Sein Ta Lone mango accessions and a mix pattern of the accessions within three locations.

INTRODUCTION

All mango varieties belong to species *Mangifera indica Linn* which is the most important economic species in the family *Anacardiaceae* [1]. Mango is originally derived from the Indo-Myanmar region during the earlier century and gradually spread to the tropical and subtropical regions of the world. Mango is a diploid fruit tree with 2n = 2x = 40 chromosomes [2]. Its genome size is approximately 4.39 × 108bp [3]. In Myanmar, mango plays a crucial role among the horticultural fruits since antiquity. The popular types of consuming mango in Myanmar are fresh dessert fruit in the ripening stage and salad in the immature stage. The global consumption of mango has increased significantly because of its great nutritional values and bioactive properties [4]. Food and Agriculture Organization (FAO) reveals that Myanmar is in the sixth rank of seven mango producing countries in ASEAN. In the future, there is the great potential to further increase the quantity of mangoes exported in Myanmar [5]. Fresh mangoes are mainly exported to China by border trade and to Singapore by overseas trade [6,7]. In Myanmar, mango is a popular fruit tree among fruit growers and can grow well in various climate conditions. In 2010-2011, it was reported that the total planted area for mango was 79,908 hectares in Myanmar and the area of fruit harvested was 70,084 hectares with total production of 503,676 metric tons. It occupied 11.85 % of total horticultural areas [8]. Mango is mainly cultivated in the central region (Mandalay and Sagaing Divisions), the southern region (Irrawaddy, Bago and Yangon Divisions), and in the east region (Southern Shan State) in Myanmar. There are about 300 varieties and 20 kinds of mango species in Myanmar and only a few cultivars such as 'Sein Ta Lone', 'Yin Kwe', 'Shwe Hin Thar', and 'Mya Kyauk' are famous in the global market for their exportable quality including high sweetness level [9]. Among them 'Sein Ta Lone' is the choicest juicy mangoes in Myanmar due to its superior characteristics such as its attractive flavor, taste, aroma, texture, pulp color and nutritional values. It is also one of the most popular and highest demands among the other commercially important fruits of global market. In Myanmar, mango has been cultivated in the 5th to 9th centuries [10], and Sein Ta Lone cultivar was cultivated in Kyaukse region more than 150 years as evident from the fact that it had 150 years old tree in Shwe Inn Phae village, Northern part of Kyaukse Township. 'Sein Ta Lone'cultivar is also regarded as the pride fruit of Kyaukse.

In the past, Mango breeders used to analyze the diversity of mangoes by using morphological features of cultivars, rootstocks, and landraces. It was a time-consuming process and easily influenced by environmental effects. DNA-based markers are versatile tools for characterizing and studying genetic similarities among land races, varieties and cultivars

[11]. Various DNA markers, including restriction fragment length polymorphism (RFLP) [12], random amplified polymorphic DNA (RAPD) Karihaloo et al, Shukla et al. [12,13], amplified fragment length polymorphism (AFLP) [14], and simple sequence repeats (SSRs) [15,16], have been utilized to determine taxonomic identity [16], to estimate genetic diversity [15], and to draw evolutionary histories of mango [14]. Among them, SSRs are widely used as a powerful tool in plant breeding programs as well as in evolutionary studies, because of their high ability for showing diversity among cultivars [17,18]. SSRs are a class of molecular markers based on tandem repeats of short (2-6bp) DNA sequences. The copy number of repeats is highly polymorphic, even among closely related genotypes. The co dominant and high polymorphic characteristics of microsatellite loci make them useful for cultivar identification [19], and hybrid evaluation [20,21]. The genetic diversity of mangoes is broadening in Myanmar due to the plenty of genetic resources of the species which are still needed to identify. However, there were a few researches based on the study of genetic variations using molecular markers. This study aims to focus on the genetic diversity and uniqueness of Myanmar mango, Sein Ta Lone cultivars which are seemed to be originated in Kyaukse region.

MATERIALS AND METHODS

Eco geographical survey and sampling

Sample collection surveys were well-planned and selected according to the recommendation of Myanmar Mango Producer Association and key informants from mango producer cooperatives. Sampling sites were conducted in different locations around Kyaukse District which include two major mangoes growing areas (Kyaukse Township and Myittha Township). In Kyaukse Township, the samples were collected from Southern part and Northern part of the Kyaukse Township. Geographical location, soil type and climate conditions of sampling area (Kyaukse District) were shown in Table 1. The mango leaves sample collection was taken during June - July 2016. The numbers of collected samples were varied according to the orchard area and the number of mango plants in its orchards. The geographic location of each of the sample tree was recorded using a hand-held global positioning system (GPS) along with the location and shown in Figure 1.

A total of 60 accessions of Myanmar Sein Ta Lone mangoes from 21 orchards in three different locations of Kyaukse District were used in this experiment and the orchard names, locations and accession codes were shown in Table 2. Random sampling strategy was followed for collection of leaf samples and the leaves were maintained in ice box to be transported from the collection sites to Molecular Genetics lab, Biotechnology Research Department, Kyaukse.

DNA extraction

Flushing, healthy and undamaged young, but fully developed collected leaves from each of the trees was washed gently with distilled water to remove all surface particles. Then the midrib of the leaves samples was removed and froze in liquid nitrogen. For molecular analysis, total genomic DNA was extracted from the leaves of Sein Ta Lone mango cultivars using the Cetyltrimethyl-

Table 1: Geographical location, soil type and climate conditions of Kyaukse District

Kyaukse District	
Location	Latitude: 21° 26′N, 22° 20′N
Longitudes:	95° 57′ E, 96°58′ E.
Area	4,147 km^2
Climate	Tropical steppe, Tropical savanna
Average temperature	maximum: 95.62°F, minimum: 66.56°F
Annual Rain fall	27.23"
Soil type	slightly acidic soil
Sample collection Date	June-July, 2016
Source: Kyaukse City Development Committee	

ammonium bromide (CTAB) method with trace modification described by Kit and Chandran. As in brief, 200mg of leaves were ground in ice cold motor and pestle mixing with 500µl of extraction buffer. The extraction buffer consisted of 3% (w/v) CTAB, 1.5 M NaCl, 1% (v/v) β-mercaptoethanol, 20 mM EDTA, 100 m MTris-HCl (pH 8.0), and 3% (w/v) PVP-40 (polyvinyl poly pyrolidone). The homogenates were incubated at 65°C for 30 min with intermittent shaking. The centrifuge tube was brought to room temperature and equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added and centrifuged at 13,000 rpm for 15 min. Then, DNA was precipitated in ice-cold ethanol by adding one-tenth volume of 3M sodium acetate. The DNA pellet was washed with 70% ethanol and dried. The dried pellet was resuspended in TE 0.1 and then treated with 10mg/ml RNase. DNA concentrations were estimated by comparison with the intensity of standard DNA (1kb ladder) after electrophoresis in 0.8% agarose gel stained with ethidium bromide. The purity and detail concentration of extracted DNA were measured by nanophotometer (Implen P330, Munich, Germany).

SSR analysis

24 primers were initially screened for polymorphism and reproducibility using Sein Ta lone cultivars. Polymerase chain reaction (PCR) amplification was accomplished in Thermal cycler (PCR-Gene Amp PCR System 9700, Singapore) under the following temperature profile: initial denaturation of 4 min at 94°C followed by 35 cycles of 1 min at 92°C, 45 sec at pairing temperature (variable according to the primers in use), 1 min at 72°C, and final extension of 5 min at 72°C. Based on the number and resolution of bands, 9 out of 24 primers were selected. Primers sequences information of 9 microsatellite markers used in this study were described in Table 3. The amplified products were separated by 8 % Polyacrylamide gel electrophoresis with 0.5 x TBE buffer at 100 V (constant voltage) for 1 hrs. The gel was stained in Silver Nitrate solution and using 0.4 M NaOH and 4% Formaldehyde as developer solution. The size of each band was determined by comparing with size standard 100 bp DNA ladder.

Data analysis

For the genetic relatedness among genotypes, all legible, unambiguously and scorable amplified fragments were arranged by binary characters with present (1) and absent (0) of the bands. Polymorphic information content (PIC) was calculated by

Table 2: Locations of mango accessions collection.

Accession Name	Number of Collected Samples	Orchard Name	Village	Township
Location 1				
SM	18	Mya Nadi	-	Myittha
SKK	2	Kay Khai	Khan Thar	Myittha
Location 2				
SB	2	Myint Gyi Monestry	Myaung OO	Northern Kyaukse
SYM	2	Sein Ya Min	Myaung OO	Northern Kyaukse
SH	3	U Htun Hla	Tha Nge Daw	Northern Kyaukse
STH	1	U Thein Han	Thin Daung Gyi	Northern Kyaukse
SHY	3	U Htun Yin	Thin Daung Gyi	Northern Kyaukse
SHK	2	Daw Hla Khin	Thin Daung Gyi	Northern Kyaukse
SHM	3	U Hla Myint	Thin Daung Gyi	Northern Kyaukse
Spu	2	Daw Puu Suu Ma	Inn Dine	Northern Kyaukse
SLp	2	Phoe La Pyae	Nga Mwe Gone	Northern Kyaukse
Location 3				
SS	3	San Da Kuu	Han Myint Mo	Southern Kyaukse
STM	3	Daw Khin Mar Mya	Han Myint Mo	Southern Kyaukse
SG	2	Mahar Aung Thapyae Monestry	Han Myint Mo	Southern Kyaukse
SCr	2	U Tin Hlaing	Khay Pin Hla	Southern Kyaukse
SKy	2	Daw Kyi Khin	Phae Khin	Southern Kyaukse
SUs	2	U Shain	Phae Khin	Southern Kyaukse
SAg	1	U Aung Khin	Phae Khin	Southern Kyaukse
SIp	1	Shwe Inn Phae	Phae Khin	Southern Kyaukse
SNy	2	U Nyo Aye	Myay So	Southern Kyaukse
SYe	2	U Kyaw Ye	Tha Pyae Gone	Southern Kyaukse
Total- 21 Orchards, 60 Samples				

(a) (b) (c)

Figure 1 Location of study area.
1. Sample collection sites in Kyaukse District
2. Mandalay Division highlighted with Kyaukse District
3. Map of Myanmar highlighted with Mandalay Division

Source: The Union of Myanmar Agriculture Atlas 2002

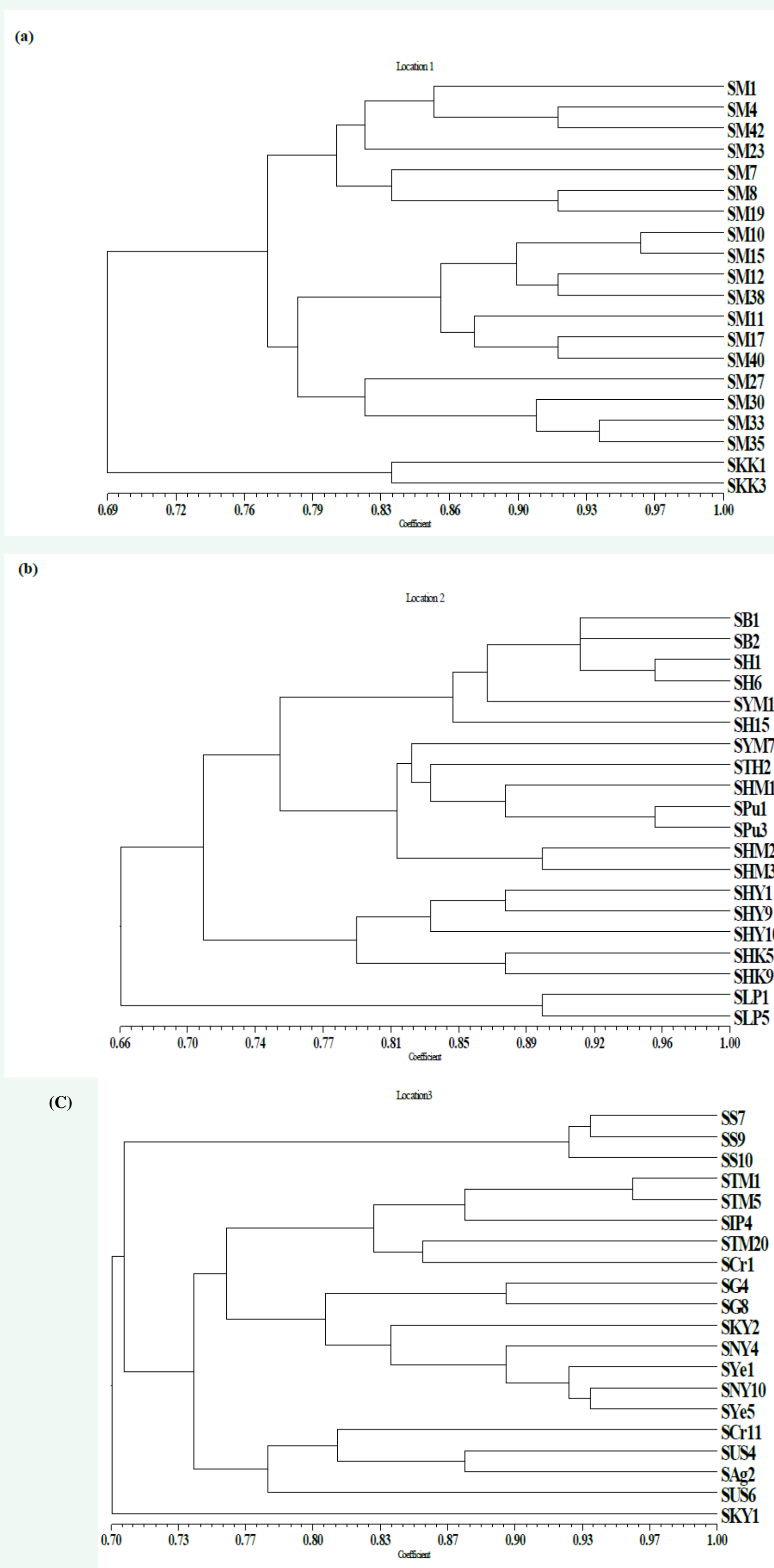

Figure 2 Dendrogram generated from SSR profile data depicting relationship among 60 Sein Ta Lone Mango accessions.
(a) Location 1 (Myittha Township)
(b) Location 2 (Northern Kyaukse Township)
(c) Location 3 (Southern Kyaukse Township)

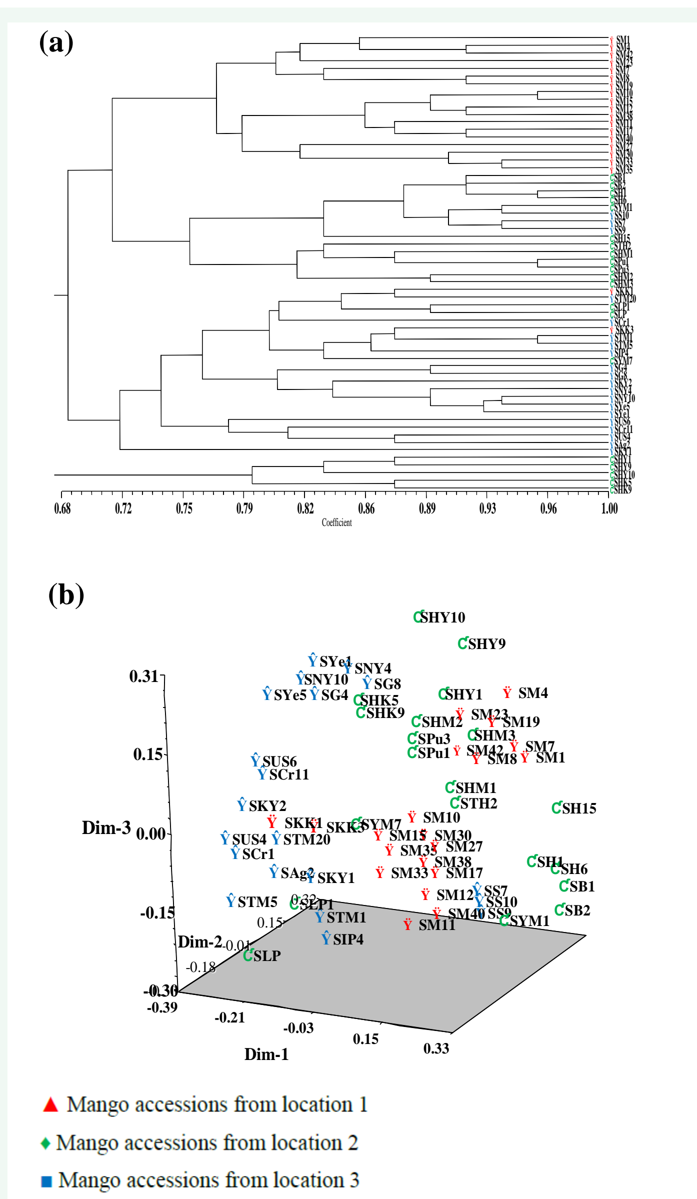

Figure 3 (a) Result of Dendrogram using UPGMA cluster analysis and (b) Principle Component Analysis based on the genetic diversity of 60 Sein Ta Lone mango accessions from Kyaukse district.

PIC= $(1-\sum Pi)^2$ as described by Nei [22]. Data analysis of Principle component analysis (PCA) and a dendrogram by means of unweight pair group method of arithmetic means (UPGMA) were constructed following the software package NTSYS pc version 2.02 (Numerical Taxonomy System) [23].

RESULTS AND DISCUSSION

Results

9 out of 24 primers were selected according to their reproducible and polymorphic DNA amplification patterns for the genetic diversity study of Sein Ta Lone mangoes. Total numbers of alleles, unbiased expected heterozygosity and polymorphic information content (PIC) by 9 SSR primers were shown in Table 4. These primers produced a total of 48 scorable bands on amplification. The polymorphism presented in the 9 microsatellite markers was ranging from 4 to 7 alleles per SSR marker and the average number of scorable loci was 5.33 loci per primer. Mn-19 primer explored the highest number of PIC value while the least polymorphism was described by MiSHR-39 primer. The unbiased expected heterozygosity (UHe) was ranging from 0.261 to 0.734 with the mean of 0.422 for each primer. In this study, all the microsatellite markers showed polymorphic information content (PIC) value (0.259 to 0.74) with an average of 0.421 per marker. The optimal annealing temperature of the 9 SSR primers was 42 to 58°C in range.

The genetic relationship of 60 mango accessions was revealed as dendrogram by using unweight pair group method of arithmetic means (UPGMA) cluster analysis method. Location 1 contained 20 Sein Ta Lone accessions from two orchards in Myittha Town ship and the UPGMA analysis of the accessions

Table 3: Microsatellite Markers used in genetic uniqueness of Myanmar Sein Ta Lone mango.

No.	Primer Name	Sequences	Size (bp)	Ta (°C)
1	LMMA -8	F- CATGGAGTTGTTGATACCTAC	257-270	45
		R- CAGAGTTAGCCATATAGAGTG		
2	MN-16	F-GCTTTATCCACATCAATATCC	150-180	42
		R-TCCTACAATAACTTGCC		
3	MiSHR-39	F-GAACGAGAAATCGGGAAC	340-369	45
		R-GCAGCCATTGAATACAGAG		
4	MN-85	F-GCTTGCTTCCAACTGAGACC	250-310	58
		R-GCAAAATGCTCGGAGAAGAC		
5	MN-84	F-TCTATAAGTGCCCCCTCACG	200-260	50
		R-ACTGCCACCGTGGAAAGTAG		
6	MN-36	F-CCTCAATCTCACTCAACA	215-245	50
		R-ACCCCACAATCAAACTAC		
7	MN-19	F-AATTATCCTATCCCTCGTATC	140-180	42
		R-AGAAACATGATGTGAACC		
8	MN-89	F-CGCCGAGCCTATAACCTCTA	110-140	50
		R-ATCATGCCCTAAACGACGAC		
9	MN-24	F-CGATGGACTTCATAAGAAGAG	150	50
		R-GCTAGCAGAATCACCTTGGTC		

Ta: Annealing Temperature

Table 4: Total number of alleles, expected heterozygosity and polymorphic information content (PIC) by 9 SSR primers.

No.	Primer Name	Alleles No.	Ho	UHe	PIC	F
1	MN-16	5	0.23	0.368	0.365	0.369
2	MN-19	4	0.5	0.734	0.74	0.318
3	MN-24	5	0.27	0.386	0.383	0.295
4	MN-36	5	0.353	0.543	0.538	0.656
5	MN-84	6	0.196	0.318	0.317	0.382
6	MN-85	6	0.323	0.445	0.441	0.267
7	MN-89	5	0.293	0.476	0.4805	0.389
8	MiSHR-39	5	0.183	0.261	0.259	0.293
9	LMMA -8	7	0.145	0.267	0.265	0.451
	Average	5.33	0.278	0.422	0.421	0.38

Ho: Observed heterozygosity
PIC: Polymorphic Information Content, $(1- \Sigma pi)^2$
UHe: Unbiased expected heterozygosity $[2N/ (2N-10] \times (1-\Sigma pi)^2$, where pi is the frequency of the ith allele of the population and N is the number of samples.
F = 1- (Ho/He)

from location 1 was shown in Figure 2a. The cluster analysis for location 1 showed that the genetic variation among the Sein Ta Lone mango genotypes was ranging from 0.69 to 0.96 similarity coefficient. Mango accessions in SKK orchard were highly diverse from all accessions in SM orchard. The similarity coefficient of two accessions from SKK orchard was 0.84.SM orchard is a large orchard under the Ministry of Agriculture and we collected 18 Sein Ta Lone accessions from this orchard. The polymorphism of accessions in SM orchard was ranging from 77% to 96% similarity. Among them, SM10 and SM15 were 96% similarity and it was predicted that they were descendant from the same parent seed.

20 mango accessions were collected from 9 orchards in Northern Kyaukse Township and regarded as location 2 and their genetic relatedness was shown in Figure 2b. The genetic dis-similarity between 9 orchards was 4% to 34%. The two mango accessions from SLp orchard were highly spread in this group with 34% dis-similarity from other orchards. The highest dis-similarity of collected samples from 4 orchards in Tha Nge Daw Village was 23%and the smallest dis-similarity was 12% within accessions from SHY and SHK orchard.

The cluster analysis of the accessions in location 3 was shown in Figure 2c. 20 samples were collected from 10 orchards in Southern Kyaukse Township. In location 3, the similarity coefficient was ranging from 0.7 to 0.96. Sky1 accession from Sky orchard was highly diverged not only from other orchards but also within its own orchard. The 3 accessions from SS orchard were also revealed as a separate group. The genetic similarities of the remaining 17 accessions were ranging from 74% to 96%. The two accessions from STM orchard shared high genetic similarity

coefficient of 0.96 and formed together.

The cluster analysis by UPGMA and the principle component analysis of 60 mango accessions were shown in Figure 3. In cluster analysis, genetic variation of all 60 accessions from 3 locations was 68% to 96 %.The UPGMA cluster analysis showed the lack of group formation according to the sampling sites. In Principle Component Analysis, it was also reinforce that there was mix spreading of location 2 and 3 although accessions from SM orchard from location 1 stand in the separated group.

Discussion

Myanmar Sein Ta Lone mango is one of the choicest cultivars of mango among the horticultural fruits not only in national level but also in global market due to its superior characteristics such as sweet taste, fibreless pulp, good aroma and flavor. The intracultivar diversity of Sein Ta Lone mangoes becomes raised and this leads to major difficulties for mango producers because of its variations in fruit size, shape and quality. Assessment of intracultivar diversity of mangoes has been made through fruit characteristics but it has many limitations because the phenotype depends on environmental and developmental factors. Vieira et al, Krishna and Singh, Kumar et al, Kalia et al. [24-27], reported that molecular characterization was more effective and unlimited by environmental and growth conditions. To the best of our knowledge, data on the regional polymorphism of Sein Ta Lone cultivar in Kyaukse District is scarce or non-existent. Kyaukse District stands out as a major producer and supplier of Sein Ta Lone cultivar because of its appropriate weather and geographical conditions. In the course of the present study, genetic diversity of Sein Ta Lone mango trees cultivated from three locations in Kyaukse District was assessed with 9 SSR markers. The 48 alleles with distinct banding patterns (alleles) were used to discriminate the Sein Ta Lone mango accessions from Kyaukse District. The PIC value of SSR markers was investigated by different researches with numbers of different markers and different genotypes. Present study showed that the average PIC value was 0.421 and it represented lower PIC value than reported by Hirano et al., Ravishankar et al., Vasugi et al., Dillon et al., [10,28-30].

The extent of genetic diversity among Sein Ta Lone accessions was studied in relation to their locations. From the base of the different locality of each accession and their clustering patterns, there was low interference (it was observed that there was not a significant correlation) between the grouping of the accessions and their locations. Rocha et al., have also reported that there was no accession that could be grouped according to collected sample locations when the genetic diversity ‘Uba’ mango tree was studied by using ISSR markers. Moreover, Karihaloo et al., and Pandit et al. [13,31], showed that mango varieties from different geographical zones in India were slightly differ in genetics and there were rare genetics relatedness about accessions grouping according to their locations of different geographical regions. Based on UPGMA clustering analysis, the genetic diversity of Sein Ta Lone mango cultivar on three locations in Kyaukse region was not quite differed. Mango accessions from SKK (Location 1), SLp orchard (Location 2) and STM orchard (Location 3) were clustered into the same group because of their close genetic similarity. There may be many other factors that can discriminate the variation of mango cultivars. Firstly, mango is cross-pollinated and orchards situated in close proximity have a less chance of pure clones. Secondly, the earlier practices of propagation by seed as well as the desire of the orchardists were equally important. Moreover, a great proportion of the commercial orchards of mangoes were asexually raised through grafting. In Kyaukse District, grafting was popular in mango producing orchards because Sein Ta Lone mango was less resistance to environmental factors and the mango producers desired to get highly resistant mango trees by grafting with other mango cultivars. This may lead to the huge genetic diversity among the Sein Ta Lone cultivar. The cluster analysis revealed that the genetic variation between Sein Ta Lone cultivar in Kyaukse district was 0.68 to 0.96 similarity coefficient and it was a huge variation within Sein Ta Lone cultivar. This study also discriminated the homogeneity and heterogeneity within the orchards. There was no clone in each orchard and the least dissimilarity was 4% in SM orchard in location 1, SH orchard and SPu orchard in location 2 and STM orchard in location3. In the present study, the analysis of genetic study by means of microsatellite markers showed high genetic diversity of Sein Ta Lone mango accessions and a mix pattern of different locations. We can also found that there was no pure clone among the Sein Ta Lone mango accessions collected from 3 locations in Kyaukse District. Therefore, the selection and maintenance of germplasm resources is also an importance factor for genetic diversity. The genetic diversity of Myanmar Sein Ta Lone Mango cultivar by means of SSR profiling studies may give some insight into the selection of genetically distinct and elite accessions of these cultivars.

CONCLUSION

The present investigation revealed the usefulness of microsatellite SSR markers in genetic diversity analysis of Sein Ta Lone mango cultivars in Kyaukse District, Myanmar. On the base of the results, it can be assumed that the location differentiation has not highly influenced the genetic diversity of Sein Ta Lone mango cultivars. Moreover, the planting of grafted mango trees to evaluate the superior phenotypic characteristics for several years may lead to the absence of 100% similarity among accessions. In the further study, it will be needed to elucidate the genetic diversity of Myanmar mango varieties from different regions of Myanmar.

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