

Collections and genetic diversity analyses of SSR markers for mango native genetic resources in Lao PDR

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Abstract

Exploration and collection of mango native genetic resources have been conducted in southern and central parts of Laos. A total of 24 accessions of mango local varieties were collected, and successfully propagated and conserved by grafting at Horticultural Research Center as the first collection of mango genetic resources in Laos. Collected genetic resources were employed for DNA analysis of SSR markers to estimate genetic relationship and diversity among accessions. From the results, it was interesting that some accessions were identified as same genotype even from different regions and different local variety names. Compared with world standard varieties, Laos mango genetic resources have potentially unique genotypes to be conserved and utilized.

Introduction

Tropical fruits are important potential cash crops for small-scale farmers in Laos and demand for these products has been increasing as a result of the economic development. Tropical fruits sell at a higher price than that of other crops, allowing farmers to reap greater profits from their limited land. Fruit trees can also be grown on the slopes that dominate much of the Laotian landscape.

Despite the market demand in Laos for tropical fruits, most are imported from neighboring countries, with domestic commercial production remaining low. Laos exported 151 tons of tropical fresh fruits in 2017, while importing 4,762 tons (the sum of the following items: [Fruit, tropical fresh nes] and [Mangoes, mangosteens, guavas] in FAOSTAT Database, <http://www.fao.org/faostat/en/#data>). This is partly due to the lack of established cultivation techniques and cultivars suitable for growth in Laos. Many tropical fruit trees in Laos are propagated by seed, resulting in varying inferior fruit quality compared with improved varieties (Dubbeldam 2005). Meanwhile, a number of fruit tree seedlings are being imported from neighboring countries such as Thailand (Boulom 2019), thus providing farmers with improved cultivars; however, the adaptability of these imported cultivars to local conditions remains unknown. Therefore, to effectively promote fruit tree cultivation in Laos, it is necessary to

evaluate the adaptability of potential commercial cultivars. Because of the limited availability of imported cultivars, an analysis of native genetic resources in Laos is of great importance.

Mango (*Mangifera indica* L.) is considered one of the most important fruit trees in Laos. In this study, we focused on genetic resources of underutilized mango trees native to Laos were collected and analyzed to provide a foundation for the promotion of domestic fruit production in Laos.

Materials and methods

Collection of native mango genetic resources in Laos

Explorations of native mango were conducted in Champasak province in southern Laos (Fig. 1A), the main fruit production area, in November 2016, and in Savannakhet and Bolikhamxai provinces (central part of Laos) in December 2017. During these visits, surveys and collections of mango genetic resources were carried out and interviews with farmers and residents were conducted in order to obtain additional information (Fig. 1B). Collected samples were propagated and conserved via grafting at the Horticulture Research Center (HRC) in Vientiane to provide a collection of mango genetic resources in Laos.

DNA analysis of diversity and phylogeny

Genomic DNA was isolated from dried mango leaves using a DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Seventeen simple sequence repeat (SSR) markers from a previous study (Yamanaka et al. 2019) were then used for PCR amplification. A phenogram was constructed using the unweighted pair-group method with arithmetic mean (UPGMA) based on the similarities between genotypes estimated by Dice's coefficient,

$$Dc = 2n_{xy} / (n_x + n_y),$$

where n_x and n_y represent the number of putative SSR alleles for materials X and Y , and n_{xy} represents the number of putative SSR alleles shared between X and Y . A phenogram was then drawn using NTSYS-pc software v. 2.1 (Rohlf 1998). To determine genetic diversity, the genetic distance between accessions was calculated from the allele size of each SSR locus using GenAIE software v. 6.5 (Peakall and Smouse 2012), followed by principal coordinates analysis (PCoA).

Results and discussion

Collection and DNA analysis of native mango genetic resources in Laos

From our survey and collection of mango genetic resources in southern and central Laos in 2016 and 2017, 24 accessions were conserved and propagated via grafting in the HRC experimental nursery (Figure 1C). These accessions were used for DNA analysis along with 7 other varieties conserved at the HRC comprising mainly imported varieties.



Fig. 1. Collection of mango native genetic resources in Laos.

A: a typical location of native mango genetic resources observed in Champasak province. Trees of local mango varieties remaining over decades were frequently found at temple gardens, public squares of villages, private backyards, so on, B: interview with farmers and residents to collect information about local mango varieties, C: conservation of collected genetic resources by grafting at the HRC.

The phenogram created based on the SSR genotypes revealed 22 identical genotypes in the 31 accessions. Moreover, accessions showing the same genotype were found in different collection areas (Figure 2). These duplicated accessions were considered the results of independent selection of common desirable characteristics in different areas (MG012 and MG042, MG015 and MG043, MG016 and MG041(MG048), and MG009 and MG040).

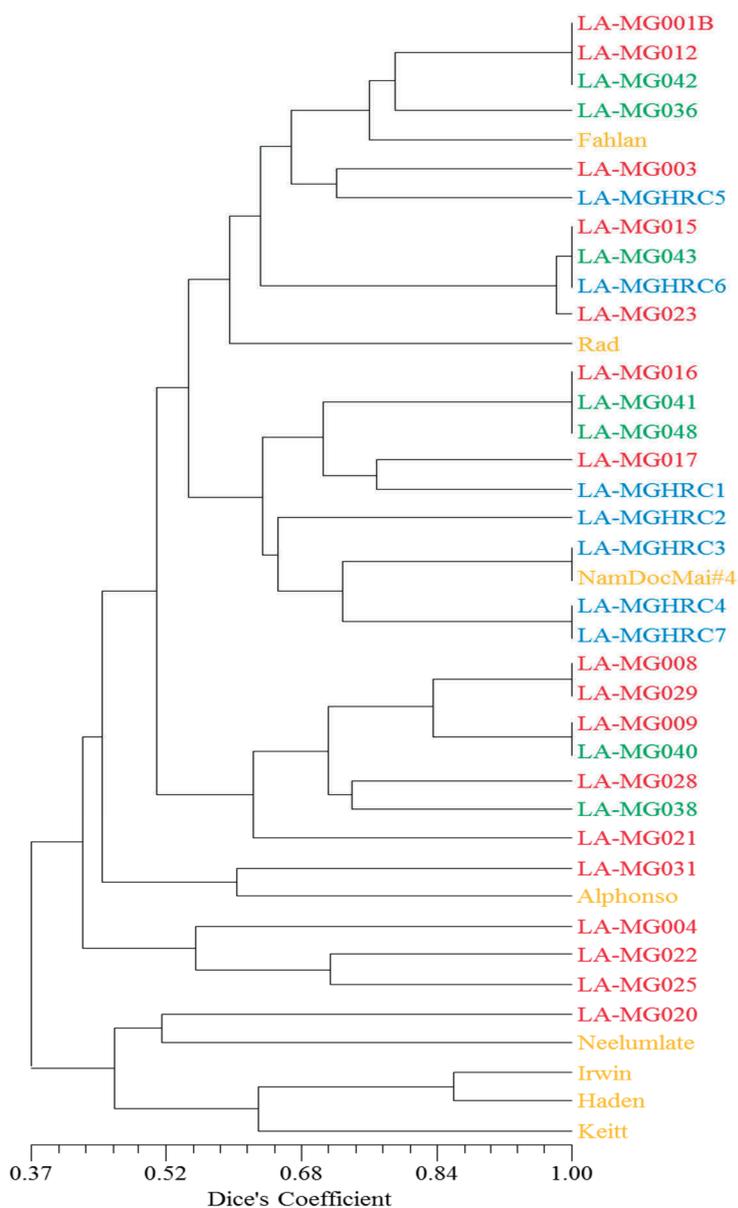


Fig. 2. Phenogram of mango native genetic resources in Laos obtained via SSR analysis. Red, green, and blue indicate the collection areas (southern and central Laos, and the HRC collections, respectively). Those highlighted in yellow represent major global varieties for comparison.

To examine genetic diversity, we also conducted PCoA using the SSR genotype data. First and second principal coordinates explained 16.88% and 15.59% of the variation, respectively, with the 31 Laos accessions distributed sparsely on the scatter plot, except for the duplicate genotypes mentioned above. These findings suggest that genetic resources in Laos possess a certain level of genetic diversity in terms of SSR variation compared with the 8 most popular global varieties (Figure 3). Further detailed analyses such as elucidation of possible pedigrees and variety identification of DNA markers are currently being conducted based on the SSR marker genotypes (Yamanaka *et al.* in preparation).

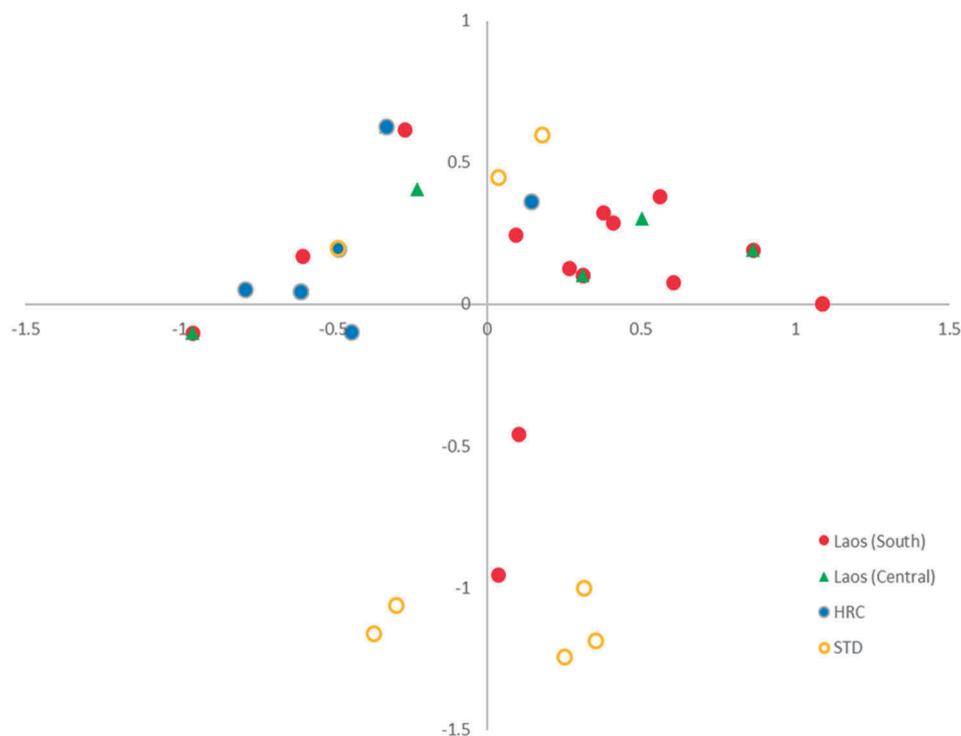


Fig. 3. Principal coordinate analysis (PCoA) of the 31 Laos accessions and 8 major global varieties (STD).

Conclusion

Mango is one of the most popular and potentially economically important fruit trees in Laos; however, at present, most fresh fruits and seedlings consumed in Laos are imported from other countries. Promotion of domestic production is therefore vital to the Laotian economy. In this study, we provided a basis for the utilization of native mango resources by collecting and conserving underutilized local varieties. In doing so, assessment of the genetic relationships between samples was carried out along with field conservation of the collected accessions at the HRC. Evaluation of important traits such as the fruit quality of these domestic mango varieties will be the subject of future research.

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References

- Boulom S (2019) Laos still reliant on imported plant supplies. *Vientiane Times* 11 July 2019
- Dubbeldam R (2005) Fruit growing as an alternative to slash-and-burn agriculture: findings and discussion. In: *Proceedings of NAFRI Workshop on "Poverty Reduction and Shifting Cultivation Stabilization*

in the Uplands of Lao PDR: Technologies, approaches and methods for improving upland Livelihoods”. National Agriculture and Forestry Research Institute, 27-30 January 2004, Luang Prabang. pp. 449–454

Food and Agriculture Organization of the United Nations (2018) FAOSTAT Database. Rome. <http://www.fao.org/faostat/en/#data>

Peakall R and Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28:2537–2539

Rohlf FJ (1998) NTSYS, numerical taxonomy and multivariate analysis system, Ver. 2.01. Exeter Publishing, Ltd., Setauket, New York

Yamanaka S, Hosaka F, Matsumura M, Onoue-Makishi Y, Nashima K, Urasaki N, Ogata T, Shoda M, Yamamoto T (2019) Genetic diversity and relatedness of mango cultivars assessed by SSR markers. *Breed Sci* 69:332–344