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Analysis of genetic polymorphisms in wild dioecious vegetable populations of *Melientha suavis* Pierre (Opiliaceae) using start codon targeted (SCoT) markers

Siringam, T. Vanijajiva, O. and Leebonoi, W.*

Faculty of Science and Technology, Phranakhon Rajabhat University, Bangkok, 10520, Thailand.

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Abstract Start Codon Targeted (SCoT) polymorphic markers were applied to assess the genetic assortment of *Melientha suavis*. Thirty informative primers were selected for their ability to generate distinct, reproducible, polymorphic bands. A total of 285 bands were observed with 58.25% showing polymorphism. The mean polymorphism information content (PIC) was 0.260. Nei's gene diversity (H) and Shannon index (I) averaged 0.047 and 0.069, respectively. Results revealed high genetic differentiation (Gst = 0.822) and gene flow (Nm = 1.108) within *M. suavis* populations. Analysis of molecular variance showed that 83% of the molecular variance was within populations. Cluster investigation, principal coordinate analysis and STRUCTURE analysis ensued separated into two distinct groups from the 47 individuals from four subdistricts in the Chai Badan district, Lopburi province of Thailand. These findings are shown to be implications for conservation programs and genotype selection in utilizing this wild vegetable plant in Thailand's deciduous forests.

Keywords: Genetic diversity, SCoT, Dioecious plant, *Melientha suavis*

Introduction

Melientha suavis, an indigenous deciduous tree endemic to Southeast Asia, commonly thrives in mixed deciduous forests and dry dipterocarp forests. It exhibits scattered distributions primarily in limestone mountain areas (Hiepko, 1984; Le *et al*., 2018). The species undergoes a flowering period that spans from March to May, during which inflorescences and fruits emerge along the stem (Figure 1). In Thailand, it occurs locally identified as "Pak Wan Pa" besides holds notable agricultural significance as a high-value vegetable crop, commanding a relatively elevated price within the region (Prathepha, 2000; Premjet *et al*., 2020). However, the prolonged harvesting period required for *M. suavis* in forested areas restricts production, necessitating more than three years for the

* **Corresponding Author:** Leebonoi, W.; **Email:** wantana@pnru.ac.th

edible parts to reach maturity (Julapak *et al*., 2016). Furthermore, the availability of this tree in the market is confined to a single annual interval, typically observed during June and July. The young leaves and stems of this tree display exceptional taste qualities and serve as essential components in various local culinary preparations (Yotapakdee *et al*., 2015). These plant parts are abundant in essential nutrients and diverse bioactive compounds, contributing to potential health benefits (Charoenchai *et al*., 2015). At present, local communities heavily depend on exploring and harvesting the species from wild populations. However, the implementation of comprehensive measures for long-term conservation and cultivation management is currently inadequate. Consequently, the natural reservoir of *M. suavis* is progressively declining, unable to sufficiently meet the growing demand. Although propagation and cultivation studies are ongoing, comprehensive genetic examinations remain constrained in their scope (Julapak *et a*l., 2016; Prathepha, 2000; Premjet *et al*., 2020).

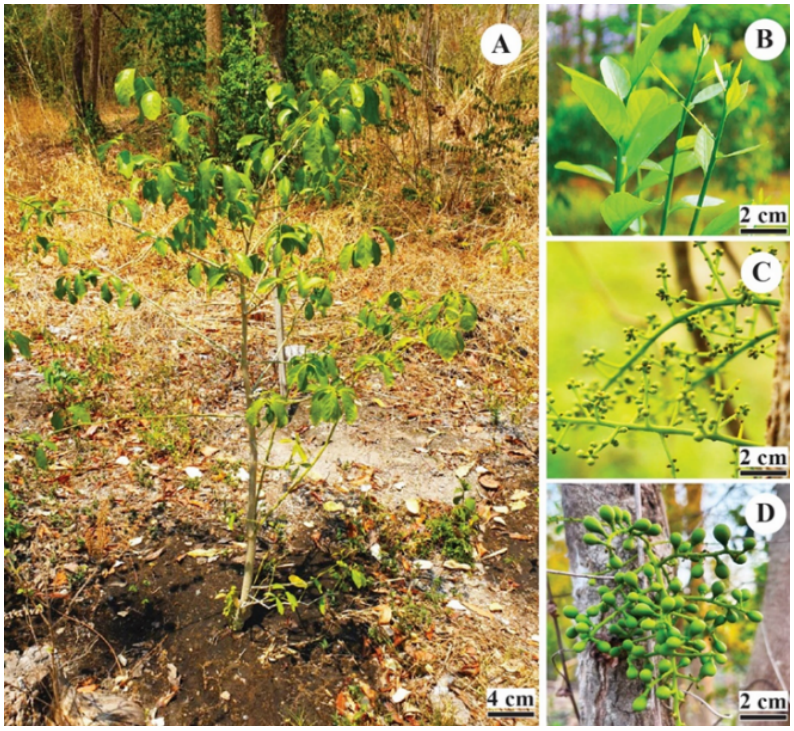

Figure 1. Plant habit (A), young leaves (B) inflorescences (B) and fruit (D) of *Melientha suavis*

Currently, modern agriculture places significant emphasis on the preservation and consumption of valuable genetic reserves. The preservation and

effective management of genetic resources are of utmost importance due to their crucial role as fundamental components in breeders' databases (Bohra *et al*., 2022). Intensely, Crop Wild Relatives (CWRs) have emerged as valuable sources of "game-changing" traits or genes that take significantly enhanced crop resistance and international cultivated production. Recent proceeds in breeding and genomics have importantly enabled the discovery of beneficial CWRs for utilization in crop advancement. This process entails a range of essential activities, including exploration, collection, characterization, evaluation, and conservation (Perrino and Wagensommer, 2022; Rankawat *et al*., 2023). Moreover, it is imperative to consider the vulnerability of wild species and primitive forms of crop plants to genetic erosion, as well as the potential consequences for agriculture (Salgotra and Chauhan, 2023). These factors underscore the potential future directions for conventional approaches, which can be further enhanced through the integration of molecular techniques (Pathirana and Carimi, 2022). In current duration, notable proceed has been designated in the event of gene-targeted indicators in plants (as reviewed by Rai, 2023). Especially, Start Codon Targeted (SCoT) polymorphisms, introduced by Collard and Mackill in 2009, are dominant and reproducible markers that rely on the conserved region surrounding the ATG start codon in plant genes. Their reproducibility is consistently observed, and it is believed that factors beyond primer length and annealing temperature contribute to their reliable amplification. The advantages of SCoT markers have been validated through studies exploring genetic diversity in numerous plant species (Shekhawat *et al*., 2018; Rai, 2023). The study established the first undertaking to utilize Start Codon Targeted (SCoT) markers in elucidating the genetic polymorphisms of vegetable wild populations of *M. suavis*. Extensive sampling was conducted within a delimited geographic region that encompasses the mixed deciduous forests of Chai Badan district in Lopburi province, Thailand, known for the thriving presence of this tree in its natural habitat. The principal aim of investigation was to measure the inherited diversity and genetic differentiation including different genotypes of *M. suavis* populations through the treatment of SCoT indicators.

Materials and methods

Plant materials

A whole of 47 samples of *M. suavis* were gathered from four subdistricts within the Chai Badan district of Lopburi province, Thailand (Table 1). All samples were taxonomically identified as belonging to the respective species

through the examination of morphological characteristics following the criteria outlined by Hiepko (1984). The living collection of all *M. suavis* specimens, along with their corresponding voucher specimens, were placed at the Chaibadanpiphat College, Phranakhon Rajabhat University, to serve as a reference for future investigations.

Table 1. Information on sample localities for all populations of *Melientha suavis*

Population code	Subdistrict	Sample site	Longitude (N)	Latitude (E)	Altitude (m)	Voucher
BM	Ban Mai Samakkhi	13	15°25'	101°05'	25-250	MS001-013
CN	Chai Narai	10	15°20'	101°06'	25-220	MS014-023
HH	Huai Hin	10	15°15'	101°04'	50-250	MS024-033
KL	Khao Laem	14	15°08'	101°00'	50-250	MS034-047

Genomic DNA extraction

Genomic DNA extraction from the leaves of 47 *M. suavis* attainments was operated using the CTAB method with slight modifications, as described by Vanijajiva (2020). The extracted DNA was further purified using the CTAB DNA extraction method, excluding the RNase treatment step. This process was continual two to three times until the DNA pellet became colorless, and the final pellet was dissolved in sterile deionized water. The characteristic of the DNA was assessed applying a Nanodrop Spectrophotometer (Thermo scientific Nanodrop 1000, USA) by measuring the absorbance ratio at 260 nm and 280 nm, which yielded a value of 1.7-1.8, indicating a pure DNA preparation. Additionally, the characteristics of the DNA were analyzed by electrophoresis on a 0.8% agarose gel using 1X TAE buffer. The extracted DNA was stored at -20 °C until further use as PCR amplification templates.

SCoT-PCR amplification

A total of thirty SCoT primers employed in this study were selected based on the methodology outlined by Collard and Mackill (2009) and underwent preliminary screening for subsequent assessment (Table 2). PCR amplification was conducted using a Thermohybaid Px2 instrument (Roche Molecular Systems, Inc., USA), with PCR conditions tailored specifically for SCoT analysis. Each PCR reaction was performed in a total volume of 20 µL, consisting of PCR buffer (Promega; 20 mM Tris-HCl, pH 8.4, 50 mM KCl), 2 mM MgCl2, 0.24 mM of each deoxyribonucleotide triphosphate, 0.5 U of Taq polymerase (Promega), and 0.8 µM of the primer. The template DNA amount in each reaction was 50 ng. The PCR protocol involved an primary denaturation phase at 94°C

for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 2 min, and a final allowance step at 72°C for 5 min. The SCoT products were subsequently examined using agarose gel electrophoresis (1.8% w/v) at 150 A for 30 minutes in 0.04 M TAE (Tris-acetate 0.001 M-EDTA) buffer at pH 8. Ethidium bromide (10 mg/ml) was used to stain the gels, which were then imagined using a Bio-Imaging System (Syngene, Genegenuis). To verify the SCoT outlines, the size of each DNA band was estimated by comparison with a 100 bp DNA ladder (Promega) employed as a molecular weight marker (M). The presence of polymorphisms at all loci was checked by performing repeat tests for each primer.

Data analysis

The present study commissioned a statistical analysis to investigate the patterns of Simple Codominant Technology (SCoT) in *M. suavis* accessions, based on several underlying assumptions. Initially, it was assumed that SCoT fragments in *M. suavis* accessions represented diploid, dominant indications, demonstrating the existence (amplified) or non-existence (non-amplified) of alleles. Furthermore, fragments that co-migrated were regarded as putatively homologous loci, and the DNA utilized in the analysis was assumed to originate from a nuclear source with biparental inheritance. Only DNA bands that exhibited reliable reproducibility were selected for further data analysis. The assessment of polymorphic information content (PIC) assessments was conducted following the methodology proposed by Roldan-Ruiz *et al.* (2000). The agroupment analysis utilized PAST 3.14 software (Hammer *et al.,* 2001) and employed the Unweighted Pair-group Method with Arithmetic Average (UPGMA) and Principal Coordinate Analysis (PCoA). The grouping of *M. suavis* accessions was created on the resulting resemblance values obtained from these analyses.

The analysis of genetic diversity parameters was done using POPGENE software, version 1.32 (Yeh *et al*., 1999). The parameters assessed encompassed Hardy-Weinberg equilibrium, the percentage of polymorphic bands (PPB), observed number of alleles (Na), effective number of alleles (Ne), Shannon index (I), and Nei's genetic diversity (H). Nei's (1987) gene diversity statistics were employed to examine genetic diversity measures, including Gst (coefficient of gene differentiation) and Nm (evaluator of gene flow), for individual populations. The hierarchical apportionment of variation was estimated using Exploration of Molecular Variance (AMOVA). Additionally, the genetic differentiation within and between populations was described using the F-

statistic (Φst) parameter (Holsinger and Weir, 2009), calculated with the ARLERQUIN program (Excoffier and Lischer, 2010).

The population's genetic structure was analyzed using STRUCTURE software version 2.3.4 (Hubisz *et al.,* 2009). The primary goal was to decide the optimal number of populations (K) in the dataset. Ten repetitions were achieved for every possibility value of K (ranging from 2 to 10). Distinct allele frequencies and genetic composition assigned individuals to clusters. Posterior probabilities, estimated through the Markov chain Monte Carlo (MCMC) method, assessed different population structures. The analysis included a burn-in period of 10,000 iterations and an additional 100,000 iterations. The model considered admixture events and correlated allele frequencies. Evanno's ΔK method (Evanno *et al.,* 2005) in STRUCTURE HARVESTER (Earl and van Holdt, 2012) determined the most suitable K value created on the rate of difference in log possibility.

Results

DNA extraction

The high-quality DNA from *M. suavis* was obtained to pose the challenges due to the presence of polyphenolic compounds in the tissues. Therefore, it is developed a high-throughput DNA removal protocol that proved to be effectively handle these challenges. The removal of high-quality DNA was modified the DNA extraction protocol by integrating re-extraction stages benefit from the CTAB DNA inaccessibility protocol and phenol:chloroform:isoamyl alcohol removal rather than chloroform:isoamyl alcohol removal. This adjustment really eradicated the polyphenolic compounds, resulting in improved SCoT electrophoretograms for all samples. The DNA isolated from *M. suavis* leaves utilizing this improved procedure showed great quality and existed appropriate for PCR effects. The minor revision technique for DNA isolation yielded clear and consistent development yields, with DNA yields ranging from 55 to 186 µg/mg of fresh weight leaf material. The A260/A280 ratios ranged from 1.78 to 1.98, indicating satisfactory DNA clarity.

SCoT polymorphism analysis

The study represented the first comprehensive investigation demonstrating significant genetic polymorphism among various *M. suavis* accessions utilizing Start Codon Targeted (SCoT) markers. In order to assess the capacity of the SCoT method to identify inherited variability at the cultivar even, the assembly was subjected to analysis using all thirty selected SCoT primers. These testing revealed distinct band repetitions for all cultivars, clearly demonstrating the

ability of the SCoT method to effectively detect genetic variation at the cultivar level. A total of 285 amplicons were generated, of which 167 bands were found to be polymorphic, resulting in a mean of 5.56 polymorphic bands per primer. The observed band lengths ranged from 100 to 1600 bp, with a polymorphism percentage of 58.07%. Among the 30 SCoT primers, SCoT15 exhibited the highest amplification, producing a maximum of 16 bands and a polymorphism percentage of 63%. Conversely, SCoT20 amplified at least 4 bands with a polymorphism percentage of 25%. Additionally, the informativeness of each primer was assessed using the polymorphic information content (PIC) criteria. (Table 2). These findings highlighted the effectiveness of SCoT markers as efficient tools for assessing within-individual and group-level genetic variability in *M. suavis*.

Discussion

Population genetic diversity and genetic differentiation

The establishment of practical protocols for SCoT analysis for molecular marker is gained significant traction in the field of population genetic variety and structure evaluation. One notable advantage of using SCoT markers is detected polymorphisms which expected to provide less biased estimations of genetic differences compared to variations at the level of gene products (Shekhawat *et al*., 2018; Rai, 2023). The findings of the study affirmed the reliability of SCoT markers as robust molecular outfit for investigating genetic difference and population formation in *M. suavis*. Furthermore, the consequences highlighted the existence of substantial genetic variation among *M. suavis* inhabitants in Thailand.

The genetic diversity and differentiation observed among species are fundamental outcomes of historical evolutionary processes, playing a crucial role in facilitating adaptation, survival, and evolution in response to dynamic environmental conditions. A substantial presence of genetic variability within a species enhanced its capacity to adapt to shifts in the environment, thereby amplifying its potential for evolutionary progress. The study presented the conclusions of comprehensive genetic assortment and differentiation considers conducted on four distinct populations of *M. suavis* located in the Chai Badan district of Lopburi province. Result provided the compelling evidence of a noteworthy level of genetic diversity exhibited by the *M. suavis* accessions. The examination of population genetic diversity revealed that the CN population exhibited the highest values for Nei's gene diversity (H = 0.073), Shannon information index (I = 0.107), and polymorphic loci (PPB = 38.25%), while the KL population displayed the lowest values for Nei's gene diversity (H = 0.017),

Shannon information index (I = 0.025), and percentage of polymorphic bands (PPB = 24.56%). Moreover, the detected and successful number of alleles were ranged from 0.860 to 1.018 and from 1.029 to 1.126, respectively. Notably, the study is revealed substantial levels of genetic differentiation (Gst = 0.822; G > 0.150 indicating high differentiation), high gene flow (Nm = 1.108; Nm > 1 indicating high gene flow), and a high fixation index (ΦST = 0.830) (Wright, 1978). Consistent with these findings, Prathepha (2000) reported similar results, highlighting elevated Gst and Nm values in their investigation of *M. suavis* utilizing RAPD markers.

Table 2. Sequence of SCoT primers and polymorphism of *Melientha suavis*

Primer	Sequences (5'–3')	Size (bp)	TAB	NPB	PPB	PIC
SCoT1	CAACAATGGCTACCACCA	320-900	9	5	55	0.245
SCoT2	CAACAATGGCTACCACCC	380-900	8	5	63	0.283
SCoT3	CAACAATGGCTACCACCG	300-810	7	5	71	0.272
SCoT4	CAACAATGGCTACCACCT	290-800	9	5	55	0.272
SCoT5	CAACAATGGCTACCACGA	300-900	9	5	55	0.256
SCoT6	CAACAATGGCTACCACGC	380-700	7	4	57	0.175
SCoT7	CAACAATGGCTACCACGG	380-800	5	4	80	0.381
SCoT8	CAACAATGGCTACCACGT	470-1,400	8	5	63	0.288
SCoT9	CAACAATGGCTACCAGCA	350-1,000	11	7	63	0.285
SCoT10	CAACAATGGCTACCAGCC	360-1,300	9	5	55	0.214
SCoT11	AAGCAATGGCTACCACCA	420-790	5	3	60	0.290
SCoT12	ACGACATGGCGACCAACG	280-1,300	9	5	55	0.251
SCoT13	ACGACATGGCGACCATCG	330-1,100	7	5	71	0.268
SCoT14	ACGACATGGCGACCACGC	300-1,400	10	7	70	0.334
SCoT15	ACGACATGGCGACCGCGA	200-1,700	16	10	63	0.280
SCoT16	ACCATGGCTACCACCGAC	250-900	9	5	55	0.237
SCoT17	ACCATGGCTACCACCGAG	220-1,300	12	7	42	0.274
SCoT18	ACCATGGCTACCACCGCC	200-900	10	6	60	0.250
SCoT19	ACCATGGCTACCACCGGC	200-1,100	13	7	54	0.247
SCoT20	ACCATGGCTACCACCGCG	800-1,100	4	1	25	0.084
SCoT21	ACGACATGGCGACCCACA	260-1,600	14	7	50	0.231
SCoT22	AACCATGGCTACCACCAC	300-1,400	11	6	55	0.264
SCoT23	CACCATGGCTACCACCAG	300-1,050	9	7	78	0.333
SCoT24	CACCATGGCTACCACCAT	340-820	10	5	50	0.207
SCoT25	ACCATGGCTACCACCGGG	180-910	10	4	40	0.180
SCoT26	ACCATGGCTACCACCGTC	280-910	11	6	55	0.213
SCoT27	ACCATGGCTACCACCGTG	180-920	9	5	55	0.267
SCoT28	CCATGGCTACCACCGCCA	100-1,000	10	7	70	0.338
SCoT29	CCATGGCTACCACCGGCC	240-880	12	6	50	0.241
SCoT30	CCATGGCTACCACCGGCG	200-790	12	8	67	0.326
total			285	166	58.25	0.260

Note: TAB = total amplified bands; NPB = number of polymorphic bands; PPB = percentage of polymorphic bands; PIC = polymorphism information content.

Table 3. Summary of genetic diversity parameters

Population	N	NPB	PPB	Na	Ne	H	I
BM	13	22	27.72	0.916 ± 0.029	1.049 ± 0.011	0.029 ± 0.006	0.044± 0.009
CN	10	52	38.25	1.018 ± 0.035	1.126 ± 0.017	0.073 ± 0.009	0.107± 0.014
HH	10	50	37.54	0.895 ± 0.040	1.112 ± 0.015	0.067 ± 0.009	0.100 ± 0.013
KL	14	13	24.56	0.860 ± 0.027	1.029 ± 0.008	0.017 ± 0.005	0.025 ± 0.007
Average		166		0.922 ± 0.017	1.079 ± 0.007	0.047 ± 0.004	0.069 ± 0.006

Note: N = number of samples; NPB = number of polymorphic bands; PPB = percentage of polymorphic bands; Na=number of different alleles; Ne = number of effective alleles; H = Nei's gene diversity index; I = Shannon's information index.

Table 4. Molecular variance (AMOVAs) for SCoT variation based on four populations of *Melientha suavis* populations sampled from Thailand

Source of variation	**df**	**Sum of squares**	**Mean squares**	**Variance components**	**Percentage of variance**	***P*-value***
Among Pops	3	347.242	8.075	8.075	17	$p<0.001$
Within Pops	43	1402.375	467.458	39.400	83	$p<0.001$
Total	46	1749.617		47.475	100	

df: Degree of freedom; P-value: probability of null hypothesis
*Significance tests after 1000 permutations

The great genetic multiplicity and variation observed in *M. suavis* are likely influenced by various factors, such as breeding systems, seed spreading mechanisms, living forms, environmental distributions, and historical origins. Notably, perennial plants with longer life histories tend to exhibit higher levels of genetic variety evaluated to their annual counterparts with shorter life cycles. As an ancient cretaceous-tertiary relict plant with an estimated evolutionary timeline of approximately 100 million years (Vidal-Russell and Nickrent, 2008; Le *et al*., 2018), *M. suavis* has undergone an extensive period of evolution, which has facilitated gene mutation, recombination, and the accumulation of genetic variations. Furthermore, the observed high gene flow in *M. suavis* can be attributed to its well-adapted nature to the limestone mountain environment, leading to the selective elimination of alleles with lower fitness.

To evaluate the genetic structure of *M. suavis* inhabitants, an analysis of molecular variance (AMOVA) was done, considering two classified levels: within and among populations. The outcomes of the AMOVA analysis revealed a substantial percentage of molecular variance within populations (83%) in comparison to among populations (17%). This finding indicated that the genetic diversity monitored among individuals within *M. suavis* populations is more pronounced than the genetic variation observed between populations. The higher

molecular variance within populations can be attributed to the outcrossing behavior characteristic of *M. suavis*, which facilitates a greater extent of gene flow within populations. This result aligns with previous investigations conducted on other dioecious woody species, such as Bartish *et al.* (1999) and Zhang *et al.* (2022), which similarly reported a high percentage of molecular variation at the intra-population intensity.

Cluster analysis

The computation of the Jaccard distance coefficient was based on binary scoring data derived from thirty SCoT polymorphic primers. It facilitated the quantification of genetic dissimilarity within a dataset comprising forty-seven genotypes representing four distinct populations. Subsequently, a cluster analysis utilizing the UPGMA method was conducted, leading to the identification of two prominent clusters.

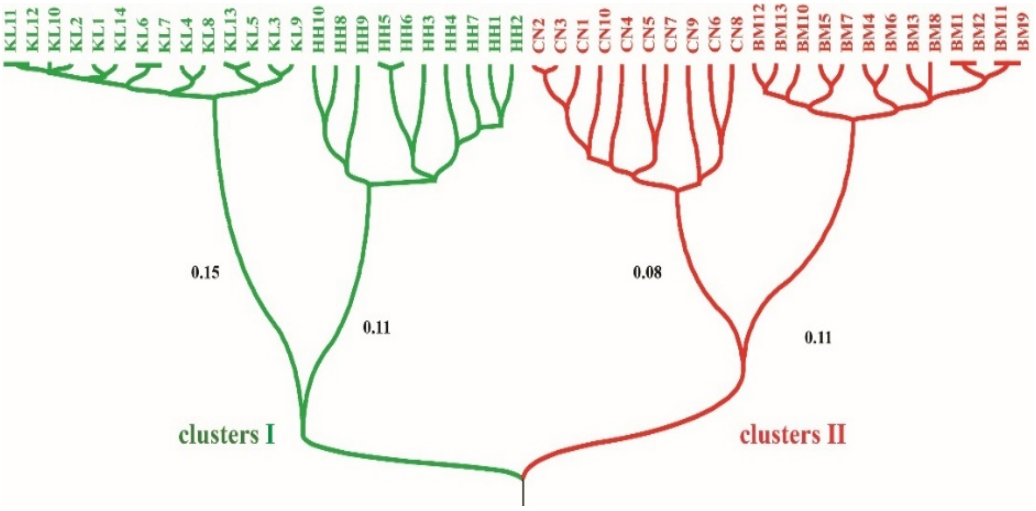

Figure 2. UPGMA dendrogram of *M. suavis* accessions

Cluster I consisted of twenty-four genotypes originating from populations located in the KL (n=14) and HH (n=10) areas. Within Cluster I, further subdivision revealed two distinct subgroups: one comprising fourteen genotypes from the KL population (KL 1–14), and the other encompassing ten genotypes from the HH population (HH 1-10). On the other hand, Cluster II comprised twenty-three genotypes derived from the BM (n=13) and CN (n=10) populations.

The resulting dendrogram provided compelling evidence that the grouping of genotypes was primarily influenced by geographical factors. Notably,

genotypes from the KL and HH populations exhibited close proximity within Cluster I, which can be attributed to their shared habitat within the mixed deciduous forests of the limestone mountain regions in the Chai Badan district, Lopburi province. Moreover, these populations demonstrated geographic adjacency, originating from neighboring subdistricts of KL and HH.

Similarly, the genotypes from the BM and CN populations, collected from open areas within the mixed deciduous forests, exhibited a pronounced association within Cluster II. This association can be explained by shared habitat characteristics and geographic proximity. Similar patterns of association have been extensively documented across various plant species inhabiting the deciduous forests of Thailand (Wattanakulpakin *et al*., 2015; Vanijajiva and Pornpongrungrueng, 2020).

The Principal Coordinate Analysis (PCoA) biplot was constructed based on the first two principal coordinates (PCo1 and PCo2), which partially validated the findings of the UPGMA clustering. As depicted in Figure 3, the PCoA results concurred with the dendrogram, thus confirming the partitioning of genotypes into two distinct clusters along the axis. Cluster I comprised all samples from the KL and HH populations, while Cluster II included the genotypes from the BM and CN populations, indicating a significant association between them. The PCoA outcomes provided compelling evidence that the grouping of genotypes was primarily influenced by geographical factors.

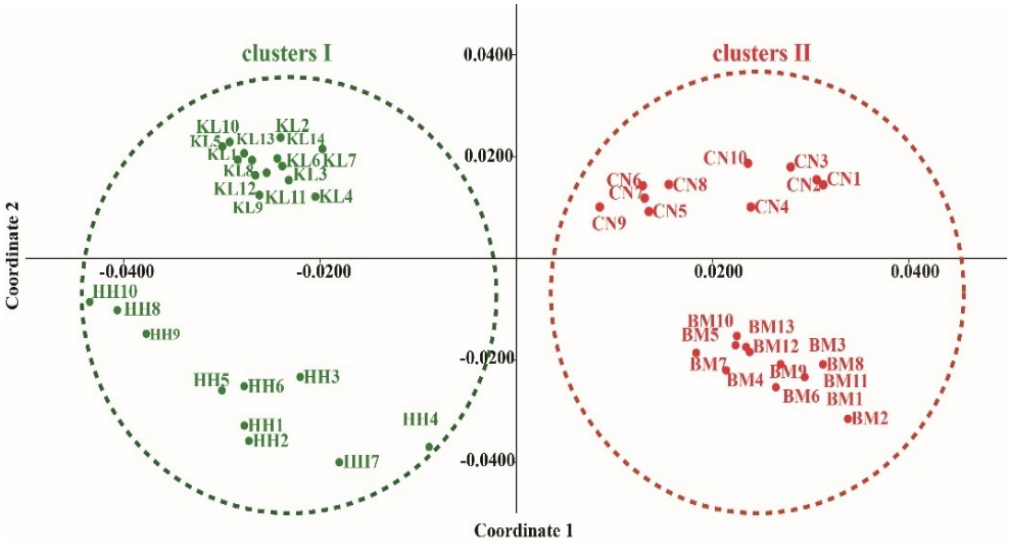

Figure 3. Principal coordinate analysis (PCoA) of *M. suavis* accessions

To more investigate the genetic correlations among populations of *M. suavis*, the inhabitant's arrangement of 47 individuals was analyzed using STRUCTURE version 2.3.4 software (Hubisz *et al.,* 2009). △K values were computed for different classes, and the analysis revealed a strong indication for $K = 2$, where $K = 2$ ideals provided the most coherent differentiation of *M. suavis* individuals across regions. Consequently, the STRUCTURE analysis classified all *M. suavis* individuals into two distinct groups. Group I consisted of individuals from the KL and HH populations, while Group II included individuals from the BM and CN populations. These findings align with the clusters identified through UPGMA and PCoA, confirming the consistency of the results across multiple analytical approaches.

The results of this study highlight the presence of substantial genetic diversity within *M. suavis* populations in the Chai Badan district of Lopburi province. Consequently, it is imperative to enhance the conservation and utilization of distinct germplasms from various populations to mitigate the risk of losing a significant number of invaluable genetic resources. Furthermore, expanding the resource collection efforts to apply transfer safety measures would facilitate more effective scientific research and resource preservation for *M. suavis*. This approach would also foster increased gene flow among populations and contribute to the overall enhancement of genetic diversity in *M. suavis.*

The utilization of SCoT analysis is offered a rapid and efficient method for characterizing *M. suavis*, and the obtained results unveiled a substantial percentage of genetic diversity within the population, along with pronounced levels of gene flow and genetic variation. Furthermore, the findings unequivocally illustrated that the association among all genotypes was primarily governed by geographic factors. Consequently, the identification of extensive genetic diversity among *M. suavis* genotypes through the implementation of SCoT markers in this investigation holds significant implications for the selection of appropriate genotypes suitable for diverse applications. Additionally, these findings can contribute to the formulation of effective conservation programs aimed at preserving this noteworthy vegetable tree species in the deciduous forests of Thailand.

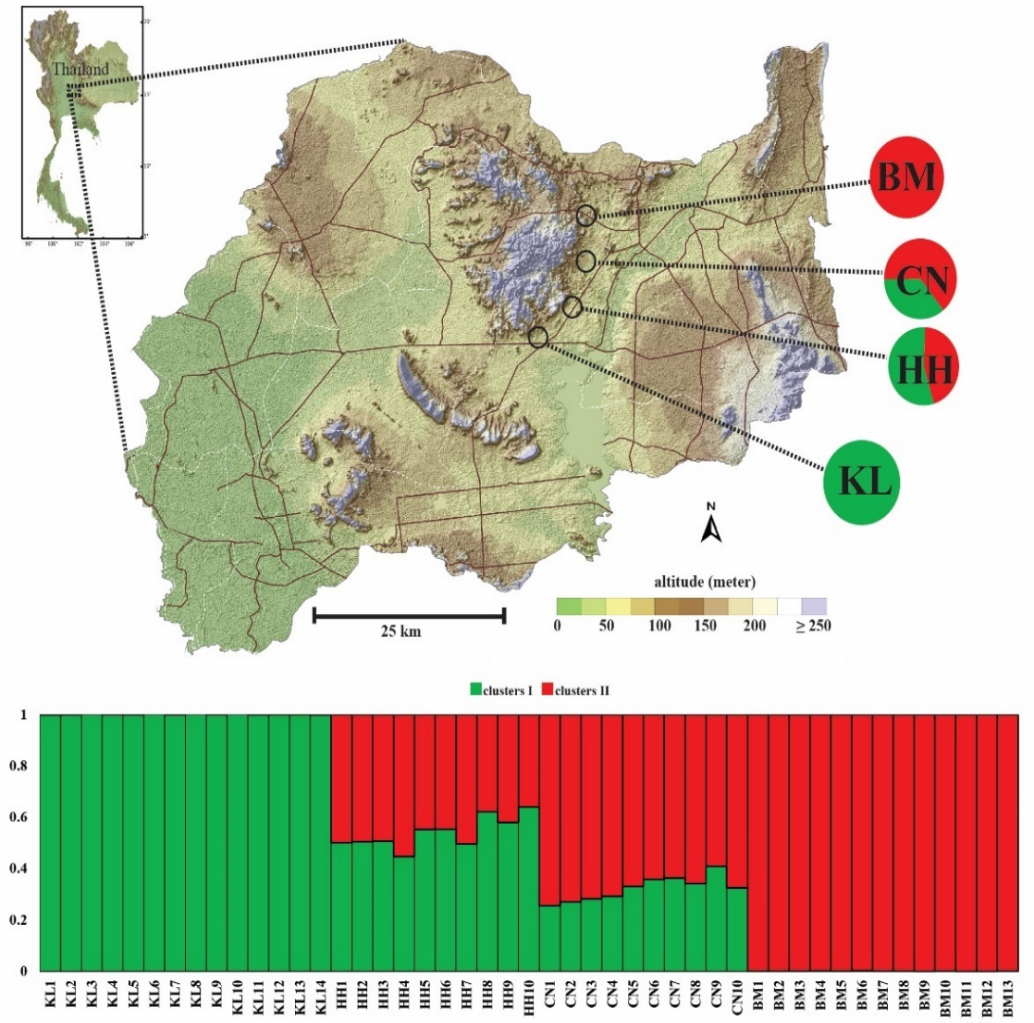

Figure 4. Scattering of the two general SCoT gene pool clusters (I and II) within and among four populations (47 individuals) of *M. suavis* as recognized by STRUCTURE based on the ad hoc statistic ΔK.

The bar-plot displays the allocation of individuals to the two groups. The y axis exhibits the expected membership coefficient (Q) for each individual in the two clusters. The x axis relates to population codes as recognized in Table 1. The pie plots on the map correspond to the average fraction of cluster membership calculated throughout population per site.

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