

Collection and evaluation of genetic diversity in melon germplasm from Central Highlands, Vietnam

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Abstract

During a collecting mission in Central Highlands (Vietnam), twenty - two landraces of melon (*Cucumis melo*) were collected. Sample information regarding their origin, cultivation, utilization and genetic variation show that at least there are three melon types in this region, “Montok” seems to be the native melon of Central Highlands, has close relationship with South and Southeast Asian melon, “Dua gang” and “Dua le” were introduced from lowland (Central coast and/or northern part of Vietnam). Montok were once widely grown by minority ethnic groups, however, the current impact of climate change and adoption of a few modern cultivars in Central Highlands poses threats to melon falling into disuse and resulting the genetic erosion. Thus, “Montok” melon in Central Highlands should be considered in conservation and evaluated for disease resistance for melon breeding purposes.

Keywords

Melon; *Cucumis melo*; Central highlands; genetic diversity, collection.

1. Introduction

Melon (*Cucumis melo* L.) is one of the most important economically crops in genus *Cucumis* which widely distributed from Old World to the New World, from Africa to Asia, Australia, Europe and America. Cultivated melons worldwide were proved to share three maternal lineages with at least two independent domestication events one in India and one in Africa (Endl et al., 2018, Zhao et al., 2019). The two different Indian lineages gave rise to spp. *agrestis* mainly distributed in East and South Asia and spp. *melo* typically grown in Europe and the United States, and African lineage gave rise to Sudanese *tibish* and *seinat* types (Zhao et al. 2019). Subsequently, India is primary centers of melon diversity; Far- East Asia and the Mediterranean regions consider as secondary centers of diversity. East Asian and Southeast Asian melons is supposed to be originated from small seed type melon in the eastern India which is the result of the eastward transmission under the pressure of wet condition (Akashi et al., 2001). Two pathways of eastward spread of melon were proposed, one is well suited to arid and semi-arid climate regions, was introduced from India to China through the “Silk Road”; another is well suited to humid climate regions, seems to have been introduced from India to Japan, Korea through Southeast Asia (Kimura, 1950).

Vietnam is located in Southeast Asia, on the route connecting India and East Asia; hence this country provided an interesting scenario for studying the domestication and diversification of Oriental melon. There are two distinct groups of the Vietnamese melon, the highland and the lowland melons (Nhi et al. 2010; Duong et al. 2021). The lowland melons are used widely as fruit-vegetable crops from north to south of Vietnam meanwhile the highland melons are restrictedly used by the ethnic minorities in the northern of Vietnam and in the Central Highlands. Central Highlands is considered as one of the biodiversity hotspots in Vietnam, high genetic diversity of crop plants and their wild related forms can be found abundantly in this region. Furthermore, Central Highlands dominated by various "tribal", especially the peoples of Malayo – Polynesian languages (Jarai and Ede) and peoples of Mon – Khmer languages (Bahnar and K'hor) which are the indigenous peoples of the southern mountainous region. Currently, the genetic base of various crops

dramatically narrows because the replacement of hybrid varieties strongly increases. Estimation of 80% of local varieties no longer existed in the crop production systems and numerous landraces are being under-studied and under-utilized (Pham et al., 2008). Therefore, the investigating of melon germplasm in Central Highlands is necessary for further breeding and conservation. The objective of this study is to collect and evaluate the genetic diversity of melon germplasm in Central Highlands with emphasis on their distribution, threats and genetic relationship with neighbor countries.

2. Methodology

Collection of germplasm

Germplasm collecting activities were undertaken on December of 2015 in Central Highlands (Figure 1). The time of collection was December, in the dry season of this region. Samples were collected from local markets and farmer houses. The collection sites were recorded using GPS. The seeds from each fruit or each farmer house were registered as one sample. The collection sample from each collection site was referred to as individual accession. We collected fruit and seed samples from local markets and farmers. The assembled information on each of the samples was surveyed are sowing date, harvest date, usage, cultivation methods. The information of melon and wild relatives was collected by interview local people and group discussion.

Molecular marker analysis

Seeds were sown on wet filter papers in petri dishes. Total DNA was extracted from young leaves of seedlings using the cetyltrimethylammonium bromide (CTAB) method, as described by Murray and Thompson (1980), with the minor modifications. The quality and quantity of each DNA sample were evaluated using Nanodrop

Nine STS-RAPD markers (A20-1100, A20-800, A22, A31, A57, B15, B71, B99-1400, B99-1550) developed by Dung et al. 2015 used in this study. The 10 μ l PCR mixture for RAPD-STS analysis comprised of 50ng of genomic DNA; 1 μ l PCR buffer (Sigma, USA: 10 mM Tris-HCl; pH 8.3, 50 mM KCl); MgCl₂; 0.1 mM dNTP; 0.25 μ M of each primer,

and 0.25U *Taq* polymerase (Sigma, USA). Amplification reactions were performed using an iCycler (Bio-Rad, USA). The PCR cycle started with an initial denaturing step at 95° C for 3 minutes, followed by 35 cycles of 1 min at 95°C, 1 min for annealing and 2 min at 72°C. The final step was at 72°C for 5 min. The annealing temperature and MgCl₂ concentration were adjusted depending on RAPD–STS primer sets (*Table 1*).

The PCR products were separated on 1.5% agarose gels in TBE buffer, stained with ethidium bromide, and visualized under ultraviolet light. For analysis the genetic relationship of melon germplasm from Central Highlands and Southeast Asian, South Asian, East Asian and Western netted melons, ten melon landraces represented of different distinct collected sites and melon types were selected. As reference accessions, 61 Southeast Asian melon accessions, consisting of 4 accessions from Indonesia, 7 accessions from Laos, 2 accessions from Malaysia, 15 accessions from Myanmar, 6 accessions from Thailand and 16 accessions of “*Dua gang*” and “*Dua le*” from Central coast of Vietnam and 30 melon accessions represented for melon genotype from South Asia, East Asia and Western netted melons were used.

Marker bands were scored as 1 for presence and 0 for absence. The output profile after scoring were used to calculate the polymorphic index content (PIC) (Botstein et al.1980; Anderson et al. 1993) and the expected heterozygosity (He) (Nei and Roychoudhury 1974), and demonstrated population structure by STRUCTURE software ver. 2.3.4.

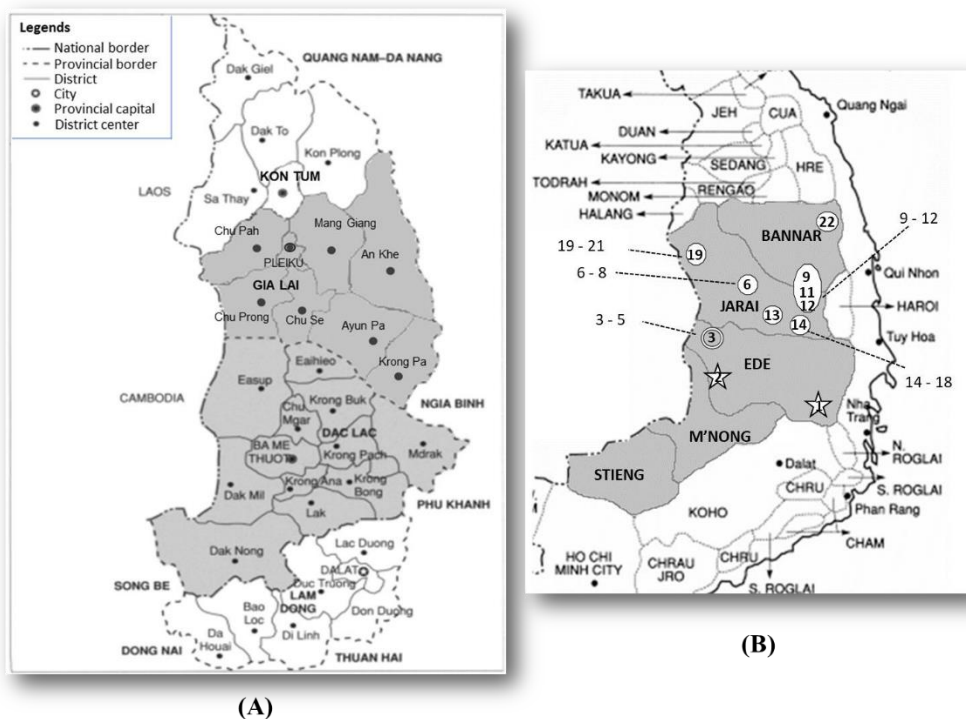


Figure 1. Maps of Central Highlands (A) and their main ethnic groups (B) (based Evans, 1992). The explored districts were indicated by grey color; each collection site is indicated by a circle symbol with indigenous ethnic group peoples of Central Highlands and by star and double circle symbol with King and H'mong peoples, respectively; the number inside the symbol indicated to accessions used for molecular marker analysis; the number outside were the ordinal number of collected accessions.

3. Results and discussion

3.1. Melon germplasm in Central Highlands

Twenty - three accessions were collected for melon during the field survey. Among them, 22 samples were local/landraces, one sample was hybrid seed. Most of melon landraces (14/22) collected from Jarai farmers in Gia Lai province. According to the Jarai farmers in Ayun Pa district, there are three types of melon, “*Montok*” – the yellow fruit with white stripe, “*Monkoo*” – the yellow fruit with no stripe, “*Monom*” – the light green fruit with no stripe, all of them could be eaten in immature and mature fruit. However, they were mixed and cultivated at the same time in the field and based on its seeds, there is no difference.

Furthermore, in the past, other type of melon called “*Monphi*” with the green fruit and white stripe was presented, this melon type could be eaten only at the mature stage because its fruit at the immature stages is very bitter, therefore, it hasn’t been preferred to be cultivated. The “*Montok*” is the common name to call melon of Jarai peoples, except for people at Ayun Pa district. One landrace melon sample was collected from Bannar farmer’s house, local name is “*Pia pong*”. These melons, “*Montok*”, “*Monkoo*”, “*Monom*”, and “*Pia pong*”, intercrop with upland rice, corn or cassava, therefore, melon season in this area is at the same time with upland rice, sown from April to June and harvested from July to September. In the cropping season from April – June to July – September, the local farmers usually stay in the cottage near the field and two or three weeks, they come back to village to get food. Therefore, melon fruit was vegetable resource; they usually eat melon fruit at the immature stage as fresh fruit like cucumber or cooking soup.

Beside the Jarai and Bannar’s melon, we also collected melon seed samples from the H’mong and Kinh peoples. However, they were non - indigenous ethnic group peoples of Central Highlands. Their settlement was the result of interprovincial population movements from the northern part under the migration policy of Vietnam. The H’mong’s farmers from whom we collected melon seeds are the second generation, therefore, they do not know the origin of seed (from the northern or from the Central Highlands); however, they call melon as “*Montok*” - same as Jarai language. The main difference in melon cultivation between Jarai and H’mong peoples is that the H’mong peoples not only grow melon in upland field but also grow in the garden. For the Kinh peoples, one “*Dua gang*” landrace (one kind of lowland melon group) was collected in this region. This type of melon was brought here in 1985 when the farmers moved from Tam ky, Quang Nam province of southern central coast region to Dac Lac province following the “New Economic program” of Vietnamese government. “*Dua gang*” is sown in December and harvested in February at the immature stage for pickled purpose. Moreover, Kinh’s farmer also used hybrid seed of “*Dua gang*” bought from seed company. Beside “*Dua gang*”, another type of Kinh’s

melon is “Dua le” also cultivated in this area, similarity with “Dua gang”, seed of “Dua le” was brought from Ninh Binh province (the northern part of Vietnam).

There is a distinct difference in cultivation method between “*Montok*” melon and lowland melons. Ethnic minorities (Jarai, Bannar, H’mong peoples) grow “*Montok*” melon intercropping with food crops (upland rice, corn, cassava...) in the rainy season (from April to October) without fertilizers and pesticide appliance. Meanwhile, Kinh peoples grow lowland melon in the dry season from February– March to April–May in the northern part and from March–April to June–July in the central part as a monoculture crop. In Central Highlands, the annual rainfall is about 2,268 mm, with the highest rainfall in August (521.5 mm). It seems to be no rain in January and February. The yearly average temperature is about 20.3°C ranging from 16.3°C to 22.5°C. Obviously, the “*Montok*” melon might adapt well with the humid condition. Adaption with humid condition was also observed in the highland melon in the northwest region (Nhi et al., 2010).

Melons of Ede people is called “*Mon*” with the similar description with “*Montok*” melon such as yellow fruit, white stripe skin, mostly immature fruit usage. Unfortunately, we could not collect this sample because of the drought in rainy season of year 2014. We couldn’t also collect any information of melon cultivation in the community of Stieng and M’ngong peoples which is located in Dac Lac province.

3.2. Genetic diversity and relationship of melon from Central Highlands

The PIC value is a quantitative measure for characterizing the degree of polymorphism in each locus (Shete, Tiwari, and Elston 2000). Based on the classification developed by Botstein et al. (1980), a PIC value is less than 0.25 indicating low polymorphism, a PIC value between 0.25 to 0.5 is average polymorphism and PIC value is higher than 0.5, that mean this is a highly polymorphic locus. Out of 9 STS-RAPD markers, 3 markers- A20-800, A57, and B99_1550 were moderate polymorphic with the PIC value ranged from 0.261 to 0.360 and 6 remained markers had low polymorphism or no polymorphism (***Table1***). The expected heterozygosity of Central Highlands melon was 0.150.

Melon from minority ethnic peoples of Central Highland was distantly related to “Dua gang”, “Dua le”. Although calling by different names, the melons collected from Jarai, Bannar and H’mong peoples did not show great variation. This type of melon grouped with melon from Southeast Asia countries, Yunnan (China), and South Asian melon (India, Nepal, Bangladesh) (**Figure 2**). This result and growing in humid condition supported that the melon group of minority ethnic people from Central Highlands of Vietnam had been transmitted from the Eastern of India. As the growing normally under humid conditions and the origin from primary centre of diversity, “Montok” could be useful genetic resources for tolerance to biotic and abiotic stresses. Therefore, this melon group needs to be paid more attention to be conserved and to be test materials for screening agricultural traits such as disease resistance. However, the role of this melon is often ignored or underestimated by crops researchers, planners and policy makers. According to Arora (2014), Neglected and Underused Species (NUS) were defined as those that communities have traditionally used for food, fiber, animal fodder, oil or for medicine, but which are seen as having further undeveloped potential uses. Most NUS crops tend to be grown by local and smallholder farmers in areas where they are still an important food source for local communities but they remain inadequately characterized and neglected by research, conservation and the economy at large scale. Considering these standards, “Montok” could be classified as under-utilized crops due to its potential value facing to the gradually lost in agricultural production and no local production plans or policies mentioned about this melon. The genetic diversity is highest is sweet melons. This may be due to the hybridization events. “Dua gang” and “Dua le” of Vietnam from Southeast Asian melon belong to Conomon group with var. *conomon* and var. *makuwa* from East Asian countries (**Figure 2** and **Table 2**).

Table 1. Primer sequence, expected size, polymorphism type and their polymorphic index content (PIC) of eight RAPD-STS for melon landraces collected from Central Highlands

RAPD-STS markers	Annealing temperature (°C)	Expected size(bp)	Type of polymorphism	PIC*
A20	62	800	InDel	0.261
		1100		0.157
A22	62	350	Presence/absence	0.087
A31	64	800	Presence/absence	0.157
A57	62	800	Presence/absence	0.360
B15	62	600	Presence/absence	0.000
B71	60	1220	Presence/absence	0.000
B99	64	1400	InDel	0.325
		1550		0.000

**Note: PIC caculated from 21 accessions (accession number 10 was not germinated for DNA extraction)*

Table 2. Common characteristics of each melon sub-population

Sub-population	Reference accession group	No. of accessions	Geographical distributi on	Genetic diversity
I	East Asian melon (var. <i>conomon</i> & var. <i>makuwa</i>)	30	Eastern Asia (Japan, China, Korea) and melon from northern central coast Vietnam	0.1052
II	Sweet melon (var. <i>inodorus</i> & var. <i>catalupensis</i>)	24	Europe, America, India, Bangladesh, Myanmar, Malaysia, Indonesia, Thailand	0.2360
III	Central Highlands of Vietnam, South Asian, Southeast Asian melon	46	India, Nepal, Bangladesh, Myanmar, Malaysia, Indonesia, Thailand, Laos, “Montok”, “Dua gang” from northern central coast and Yunnan (China)	0.1311

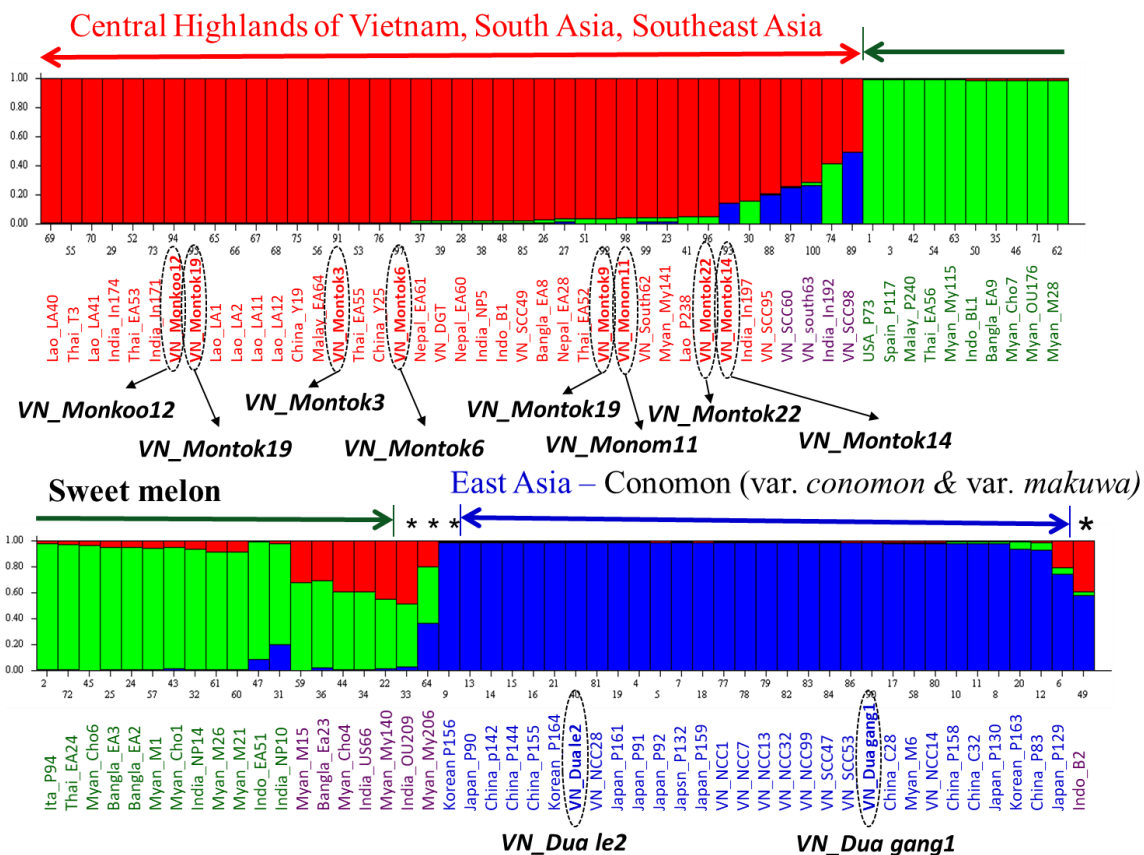


Figure 2. Model-based cluster membership of 100 accessions into three sub-populations identified with STRUCTURE using nine RAPD-STs markers. Ten accessions represented to melon from Central Highlands were indicated by dot elliptic, the code of these melon included 3 parts, VN_<local name><accession number>

4. Conclusion

In Central Highlands of Vietnam, there are three melon types which were divided into two melon population, “Montok” and Vietnamese conomon. “Montok” seems to be the native melon of this region and had been transmitted from the Eastern of India. As the growing normally under humid conditions and the origin from primary centre of diversity, “Montok” could be useful genetic resources for tolerance to biotic and abiotic stresses. Vietnamese conomon, “Dua gang” and “Dua le”, was introduced from lowland (Central

coast and/or northern part of Vietnam) and grouped with var. *conomon* and var. *makuwa* from East Asian countries.

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REFERENCES

1. Anderson, J. A., G.A. Churchill, J.E. Autrique, S.D. Tanksley, and M.E. Sorrells (1993) Optimizing parental selection for genetic linkage maps. *Genome* 36: 181–186.
2. Akashi, Y., N. Fukuda, T. Wako, M. Masuda and K. Kato (2002) Genetic variation and phylogenetic relationships in East and South Asian melons, *Cucumis melo* L., based on the analysis of five isozymes. *Euphytica* 125: 385–396.
3. Arora, R. K. (2014). Diversity in underutilized plant species: An Asia-Pacific perspective. *Biodiversity international*.
4. Botstein, D., R.L. White, M. Skolnick, and R.W. Davis (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314–331.
5. Dung, T. P., Thuy, D. T., Tanaka, K., Nhi, P. T. P., Nishida, H., & Kato, K. (2015). Development of RAPD-Derived STS Markers for Genetic Diversity Assessment in Melon (*Cucumis melo* L.). *Hue University Journal of Science (HUJOS)*, 114(15), 449-456.
6. Duong, T. T., Dung, T. P., Tanaka, K., Nhi, P. T. P., Shigita, G., Imoh, O. N., ... & Kato, K. (2021). Distribution of two groups of melon landraces and inter-group hybridization enhanced genetic diversity in Vietnam. *Breeding Science*, 20090.
7. Endl, J., E.G. Achigan-Dako, A.K. Pandey, A.J. Monforte, B. Pico, H. Schaefer (2018) Repeated domestication of melon (*Cucumis melo*) in Africa and Asia and a new close relative from India. *American Journal of Botany* 105:1662–1671.

8. Evans, G. (1992). Internal Colonialism in the Central Highlands of Vietnam. *Journal of Social Issues in Southeast Asia* 7 (2), 274-304.
9. Kitamura, S. (1950) Notes on Cucumis of Far East. *Acta. Phytotax et Geobot.* 14: 41–44.
10. Murray, M.G. and W.F. Thompson (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8: 4321–4326.
11. Nei, M. and A.K. Roychoudhury (1974) Sampling variances of heterozygosity and genetic distance. *Genetics.* 76: 379–390.
12. Nhi, P.T.P., Y. Akashi, T.T.M. Hang, K. Tanaka, Y. Aierken, T. Yamamoto, H. Nishida, C. Long, and K. Kato (2010) Genetic diversity in Vietnamese melon landraces revealed by the analyses of morphological traits and nuclear and cytoplasmic molecular markers. *Breed. Sci.* 60: 255–266.
13. Shete, S., Tiwari, H., & Elston, R. C. (2000). On estimating the heterozygosity and polymorphism information content value. *Theoretical population biology*, 57(3), 265-271.
14. Zhao, G., Q. Lian, Z. Zhang, Q. Fu, Y. He, S. Ma, V. Ruggieri, A. J. Monforte, P. Wang, I. Julca, et al.. (2019) A comprehensive genome variation map of melon identifies multiple domestication events and loci influencing agronomic traits. *Nature Genetics*, 51(11): 1607– 1615.



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