
Phenotypic diversity and classification of Thai bitter melon (*Momordica charantia* L.) landraces from three provinces in central region of Thailand

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Seventy four (74) accessions of Thai bitter melon (*Momordica charantia* L.) landraces were collected from three provinces in central region of Thailand including Prajuabkirikhan, Samutprakarn and Chachoengsao, and phenotypic diversity in horticultural characteristics was assessed. They showed rather high phenotypic variation in fruit weight and seeds per fruit, and appreciable variation in several traits such as petiole length, petal width of both staminate and pistillate flower, fruit width, fruit length and fruit peduncle length. Principal component analysis of 15 representative characteristics was performed to determine the most important variables contributing to the variation. It revealed that the first 7 principal components (Eigen values > 1) accounted for 69.82 % of the total multivariate variation among the accessions. Hierarchical cluster analysis based on Ward's distance method was conducted using the 7 components; it grouped the 74 accessions into four appropriate clusters at the level of 20 squared euclidean distances. This classification is useful for breeders to identify accessions with desirable characteristics for breeding programs.

Key words: Thai bitter melon, phenotypic diversity, PCA, cluster analysis

Introduction

Bitter melon, bitter gourd, bitter cucumber or balsam pear grows commonly throughout Thailand, and is grown as a vegetable crop all over the tropics, including South East Asia, India, South America, East Africa and the Caribbean (Morgan and Midmore, 2002). The immature fruits and the tender leafy shoots or the ripe fruits (Yamaguchi, 1983) have both nutritional as well as medicinal importance (Khan and Anderson, 2003). It has a long history of medicinal use especially in a diabetes treatment and other medicinal properties

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(Robinson and Decker, 1997; Abascal and Yarnell, 2008). Bitter melon grown in Thailand could be divided into two types; one type has large and cylindrical fruit, which is usually called Chinese bitter melon or Mara-chin. The other has much smaller and pear-shaped fruit, which is known as Thai bitter melon or Mara-Khee-Nok. Thai bitter melon may have low market share in comparison to Chinese bitter melon; however, it has played important roles both in sufficient economy for rural Thai people and in Thai herbal medicine for a long time. Since it is a cross-pollinated crop, and farmers usually maintain their own seeds from season to season, thus there might be variations in most traits of landraces. The traditional landraces are the important genetic resources for plant breeders because of their considerable genotypic variations. These variations might be maintained by deliberate selection for specific traits by farmers. Determination of the degree of variation of quantitative and qualitative traits presenting in genetic resources is important for vegetable breeding programs (Escribano *et al.*, 1998).

Principal component analysis (PCA) is useful in analyzing genetic variation among plant accessions and determining the most important variables contributing to this variation, as used by Shankar *et al.* (2009) in diversity analysis of forty six bitter melon landraces in India. In the current study, seventy four accessions of Thai bitter melon landrace collected from three provinces in central Thailand were analyzed for phenotypic traits using PCA, and then they were grouped by cluster analysis based on principal components from PCA. This is an important step in evaluating the materials as genetic resources for breeding programs.

Materials and methods

Seeds of Thai bitter melon landraces were collected from Prajuabkirikhan (16 accessions), Samutprakarn (31 accessions) and Chachoengsao (27 accessions) provinces, central region of Thailand. Seeds were firstly sown in plug trays on 18 December 2008 and then seedlings were transplanted to field plots. Because of poor seed germination, only 74 from original 100 accessions sown could be grown and observed their characteristics. Four seedlings from each accession were field planted at the two - three leaf stage at a spacing of 0.50 x 0.75 m. Bamboo poles were used as a trellis to support the climbing vine. Standard fertilization, weed control and other cultural practices were applied to raise a good crop. Data were recorded on horticultural characteristics such as leaf size, male and female flowers, fruit (ripe) size and weight, seed size and number of seeds per fruit. Values averaged from ten samples of each accession were subjected to further evaluation. Statistical analysis was performed using the statistical package SPSS (14.0 for Windows). The study

was conducted at Faculty of Agriculture and Natural Resources, Rajamangala University of Technology Tawan-ok, Chonburi province, Thailand.

Data analysis

Twenty six phenotypic characteristics were analyzed using descriptive statistics to indicate their variations. To make more accurate calculation, only fifteen characteristics were chosen to principal component analysis (PCA). In PCA, the data were performed standardization, then were used to generate Eigen values, the percentage of the variation accumulated by PCA and the load coefficient values which relate the values (eigen values are proportional to the amount of total variation among the accessions that is associated with the axis). Those principal components (PCs) with Eigen values >1.0 were selected and those characters with load the highest coefficient values were considered highly relevant for each PC. Factor scores of those PCs were subjected to cluster analysis. Hierarchical cluster analysis was performed using Ward's method, which was preferred because it tends to produce desirable compact clusters (Zewdie and Zeven, 1997).

Results

The minimum, maximum, mean values, standard deviations (SD) and coefficients of variation (CV) in twenty six characteristics are shown in Table 1. The results revealed a large variation among accessions in some traits. For leaf character, petiole length was the highest variation as depicted by CV values. In terms of staminate and pistillate flowers, petal width had more variation than petal length, and sessile bract length was more diverse than its width. For most flower traits, male flower had higher variation than female flower. In view of fruit character, all characteristics were highly diverse except days to anthesis. For seed size, seed thickness had more variation than seed width and length. In consideration of all traits, fruit weight and seeds per fruit were clearly high in diversity as they had high CV values of 40.82 and 40.87 per cent, respectively.

PCA of fifteen traits associated with seventy four accessions of Thai bitter melon landrace is shown in Table 2. The fifteen traits were reduced to seven variables (components), which responded 69.82 % of the total variation. The principal component 1 (PC1) had the largest Eigen-value (1.950) and accounted for the greatest amount of variance in the original data. Characters with higher coefficients on the PCs should be considered more important, thus higher eigen vectors are shown in bold in Table 2. Fruit length and seeds per fruit had higher

coefficients of the first PC than on the others. The other principal components could be explained in a similar fashion.

Using the seven PCs, hierarchical cluster analysis was performed based on Ward's distance method. The analysis grouped the 74 accessions into four clusters at the level of 20 squared euclidean distances. The means and standard deviations for the traits for each cluster are presented in Table 3. The four clusters are described as follows.

Table 1. Variability of some traits of 74 local Thai bitter melon accessions.

Traits	Min.	Max.	Mean	SD	CV (%)
Leaf					
Width (cm)	6.90	9.82	8.17	0.578	7.07
Length (cm)	5.84	8.25	6.95	0.543	7.81
Petiole length (cm)	3.75	8.21	5.74	0.962	16.76
Staminate flower					
Petal width (cm)	0.68	1.79	1.18	0.242	20.51
Petal length (cm)	1.23	2.08	1.68	0.170	10.12
Peduncle length (cm)	8.42	12.53	10.76	0.570	5.30
Sessile bract width (cm)	0.68	1.90	1.43	0.221	15.45
Sessile bract length (cm)	0.61	2.43	1.07	0.250	23.36
Days to anthesis	49.00	64.00	56.07	3.763	6.71
Pistillate flower					
Petal width (cm)	0.82	1.77	1.31	0.205	15.65
Petal length (cm)	0.98	2.08	1.748	0.181	10.35
Peduncle length (cm)	8.60	11.64	10.46	0.471	4.50
Sessile bract width (cm)	1.08	1.72	1.44	0.127	8.82
Sessile bract length (cm)	0.74	1.52	1.09	0.145	13.30
Days to anthesis	48.00	64.00	55.82	3.956	7.09
Fruit (ripe)					
Width (cm)	1.70	4.00	2.92	0.522	17.88
Length (cm)	4.00	9.00	6.09	1.218	20.00
Peduncle length (cm)	4.60	14.50	8.64	2.435	28.18
Cavity width (cm)	1.00	3.00	1.95	0.458	23.49
Cavity length (cm)	2.20	7.00	4.49	1.051	23.41
Weight (g)	3.80	18.20	7.58	3.094	40.82
Days to first harvest	68.00	79.00	72.96	2.706	3.71
Seed					
Width (cm)	0.44	0.63	0.52	0.030	5.77
Length (cm)	0.90	1.07	0.99	0.038	3.84
Thickness (cm)	0.14	0.26	0.20	0.025	12.50
Seeds per fruit	3.00	27.00	10.85	4.434	40.87

Table 2. Principal component analysis (PCA) of traits associated with 74 accessions of Thai bitter melon landrace. Proportions of variations are associated with first seven components, which correspond to Eigen values greater than 1.

Principal component analysis	Components						
	1	2	3	4	5	6	7
Eigen values	1.950	1.633	1.570	1.525	1.453	1.215	1.127
Explained proportion of variation (%)	13.00	10.89	10.47	10.17	9.68	8.10	7.52
Cumulative proportion of variation (%)	13.00	23.89	34.36	44.52	54.21	62.31	69.82
Traits	Eigen vectors						
Fruit length	0.85						
Seeds per fruit	0.77						
Petiole length		0.75					
Leaf length	0.29	0.73					
Petal length ¹			0.77		0.30		0.23
Sessile bract length ¹			0.64				
Peduncle length ¹	-0.48		-0.58			-0.21	0.27
Fruit weight				0.79			
Seed length		0.22		0.79			
Seed width					-0.78		0.23
Sessile bract length ²	0.28			0.32	0.59	-0.26	
Petal length ²	0.39	-0.21	-0.41		0.55		
Peduncle length ²		-0.27				0.80	
Leaf width		0.51		0.25		0.57	
Fruit peduncle length							-0.93

¹Staminate flower, ²Pistillate flower.

Cluster A composed of 22 accessions which had a quite large leaf with an average leaf width of 8.49 cm, leaf length of 7.09 cm and petiole length of 6.29 cm. Fruits were quite small with a length of 5.83 cm and a weight of 6.97 g. This group was also predominant in large staminate flower which had an average petal length of 1.80 cm.

Cluster B included 17 accessions which had quite small pistillate flower with an average petal length of 1.63 cm. Fruits were also quite small with a length of 6.22 cm and a weight of 6.93 g. Cluster C was the largest group containing 24 accessions. This group was moderate in fruit weight with an average of 7.55 g. Average number of seeds per fruit was 9.79 seeds, which was ranked fourth. The smallest group was cluster D which was composed of 11 accessions. This group was predominant in a large fruit with an

Table 3. Means and standard deviations (in parentheses) of the four clusters of 74 local Thai bitter melon accessions classified by cluster analysis according to the seven PCs from principle component analysis.

Traits ¹	Cluster A	Cluster B	Cluster C	Cluster D
Number of accessions	22	17	24	11
Leaf				
Width	8.49 (0.65)	7.87 (0.50)	8.10 (0.44)	8.14 (0.57)
Length	7.09 (0.57)	7.01 (0.50)	6.75 (0.55)	6.98 (0.49)
Petiole length	6.29 (1.07)	5.65 (1.01)	5.40 (0.61)	5.50 (0.92)
Staminate flower				
Petal length	1.80 (0.13)	1.60 (0.18)	1.64 (0.16)	1.63 (0.11)
Peduncle length	10.50 (0.67)	10.72 (0.34)	11.06 (0.53)	10.66 (0.46)
Sessile bract length	1.27 (0.32)	1.10 (0.10)	0.90 (0.14)	0.98 (0.12)
Pistillate flower				
Petal length	1.68 (0.13)	1.63 (0.23)	1.81 (0.12)	1.93 (0.10)
Peduncle length	10.51 (0.42)	10.19 (0.62)	10.56 (0.38)	10.55 (0.36)
Sessile bract length	1.09 (0.13)	1.04 (0.15)	1.08 (0.15)	1.21 (0.10)
Fruit (ripe)				
Length	5.83 (0.99)	6.22 (1.46)	5.76 (1.01)	7.11 (1.18)
Peduncle length	7.86 (2.07)	9.88 (2.01)	7.07 (1.36)	11.73 (1.79)
Weight	6.97 (1.90)	6.93 (3.57)	7.55 (2.53)	9.89 (4.46)
Seed				
Width	0.51 (0.02)	0.55 (0.03)	0.52 (0.01)	0.48 (0.03)
Length	0.99 (0.05)	0.99 (0.03)	0.98 (0.03)	1.00 (0.04)
Seeds per fruit	10.23 (3.21)	11.76 (5.68)	9.79 (3.31)	13.00 (5.92)

¹ Width and length units are in centimeter, weight in gram.

Average in fruit length of 7.11 cm and fruit weight of 9.89 cm. In addition, petal length of pistillate flower averaged 1.93 cm, which ranked it first for all groups. It was also the first ranking in number of seeds per fruit with an average of 13.00 seeds.

Discussion

Thai bitter melon has been reported as one of the under-utilized vegetables in Thailand; even it was well-known and often consumed by Thai people especially in rural areas. It was suggested that a research should emphasize its potential as a health food vegetable and as a medicinal plant (Nath *et al.*, 1999), as the previous work conducted by Ditchaiwong *et al.* (2002). This current study focusing germplasm collection is a primary step for the breeding program. To make more understanding of these genetic resources for future breeding efforts, it is significant to evaluate the most useful characters to define groups. Presently, multiple traits analyses such as principal

component analysis and cluster analysis have been used for the evaluation of plant diversity.

In this study, phenotypic diversity within Thai bitter melon accessions was high in several characteristics; however, the previous reports which were performed in the populations randomly derived from these 74 accessions indicated that environmental factors highly affected on the inheritance of traits (Numuen and Pornsuriya, 2010; Pornsuriya *at al.*, 2010).

Cluster analysis grouped the 74 accessions into four clusters. Each cluster had remarkable characteristics that would be useful for plant breeders in order to select desirable materials. In addition, each cluster contained accessions from two or three provinces, implying that variations might not depend on provincial geography.

PCA and cluster analysis should be accounted as the powerful methods in managing multiple traits for plant breeding, especially in landrace populations. They could define many traits into few factors, and then cluster plant accessions into groups, that is convenient for plant breeders to consider the information and use in breeding programs.

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