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GENETICS DIVERSITY BASED ON AGRICULTURAL TRAITS AND PHYTOCHEMICAL CONTENTS IN SPINY BITTER GOURD (*Momordica cochinchinensis* (Lour.) Spreng)

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SUMMARY

Spiny bitter gourd is a highly nutritious plant as its aril contains high contents of phytochemicals, especially -lycopene and beta carotene. The information on genetic variation in germplasm is important for development of new varieties. The objective of this study was to evaluate the genetic diversity of spiny bitter gourd based on agricultural traits and phytochemical contents. Twenty-six accessions of spiny bitter gourd collected from different parts of Thailand and Vietnam were evaluated in a randomized complete block design with 3 replications at the Fruit Orchard Research Farm, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand during July to October 2010. Genetic diversity of 26 accessions of spiny bitter gourd was identified based on horticultural traits (fruit fresh weight, aril weight, days to fruit maturity and number of seeds) and phytochemical contents (total carotenoids, lycopene, beta-carotene and lycopene per fruit). High variations were observed in these accessions for all agricultural traits and phytochemical contents. Cluster analysis showed that the 26 accessions of spiny bitter gourd could be grouped into 6 distinct clusters. The results are useful for germplasm conservation, utilization and management for breeding of spiny bitter gourd in the future.

Key words: Breeding, cluster analysis, gac fruit, germplasm, lycopene, beta carotene.

Key findings: Grouping of germplasm into different clusters using agricultural traits and level of phytochemical contents was a mean to identify genetic differences. This knowledge will enable useful information for germplasm conservation, utilization and management for spiny bitter gourd breeders.

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INTRODUCTION

Spiny bitter gourd (*Momordica cochinchinensis* (Lour.) Spreng) is an underutilized tropical vegetable crop in Asia. It's also called baby jackfruit, Gac fruit, sweet gourd, cochinchin gourd. The crop is known under different names in different countries such as Gac in Vietnam, Fak kao in Thailand, Bhat Kerala in India, Moc Niet Tu in China and Mak kao in Laos. It is a member of perennial dioecious cucurbit family (Sanwal *et al.,* 2011). Spiny bitter gourd has long been used as a food and traditional medicine in East and Southeast Asia (Kubola and Siriamornpun, 2011). Placenta or aril (seed membrane) is an excellent source of bio-accessible carotenoids (lycopene and beta-carotene) (Vuong *et al.,* 2006; Burke *et al.,* 2005). These phytochemicals are beneficial to health as they can reduce the risk of several diseases such as prostate cancer, colon cancer, stomach cancer and cerebral thrombosis (Voung *et al.,* 2006; Ishida *et al.,* 2009). In addition, spiny bitter gourd oil has been readily accepted by women and children of Vietnam, and consumption of the oil can reduce lard intake (Vuong *et al.,* 2002; Vuong and King, 2003; Ishida *et al.,* 2004).

The potential uses of this underutilized crop are as raw material for natural colorant industry, food additive and functional food products because of its high carotenoid content in placenta or seed membrane. Breeding for high and stable carotenoid content and yield is important for industrial utilization of spiny bitter gourd, and the information on genetic diversity among germplasm is also important for effective breeding programs. Unfortunately, this useful information is not available in the literature. The objective of this study was to determine genetic diversity and genetic relatedness among 26 landraces of spiny bitter gourd accessions collected in Thailand and Vietnam using agricultural traits and phytochemical contents. The information is very useful for genetic improvement of this crop.

MATERIALS AND METHODS

Plant material

The 26 spiny bitter gourd accessions were evaluated in study. Twenty-five accessions were collected in Thailand and 1 accession was collected from Vietnam (Table 1).

Table 1. List of spiny bitter gourd accessions used in this study and sites of collection.

Accessions	Collection sites
KKU ac.09-002	Khon Kaen Province, Thailand
KKU ac.09-003	Khon Kaen Province, Thailand
KKU ac.09-004	Khon Kaen Province, Thailand
KKU ac.09-008	Chiang Mai Province, Thailand
KKU ac.09-010	Nakhon Pathom Province, Thailand
KKU ac.09-011	Nong Khai Province, Thailand
KKU ac.09-012	Kalasin Province, Thailand
KKU ac.09-013	Kalasin Province, Thailand
KKU ac.09-016	Khon Kaen Province, Thailand
KKU ac.09-018	Yasothon Province, Thailand
KKU ac.09-019	Hybrid#3
KKU ac.09-020	Hybrid#4
KKU ac.09-027	Kalasin Province, Thailand
KKU ac.09-030	Kalasin Province, Thailand
KKU ac.09-033	Kanchanaburi Province, Thailand
KKU ac.09-034	Chaiyaphum Province, Thailand
KKU ac.10-043	Hybrid#10
KKU ac.10-049	Khon Kaen Province, Thailand
KKU ac.10-077	Hybrid#40
KKU ac.10-080	Hybrid#43
KKU ac.10-086	Hybrid#48
KKU ac.10-087	Nakhon Pathom Province, Thailand
KKU ac.10-090	Samut Songkhram Province, Thailand
KKU ac.10-094	Vietnam
KKU ac.11-138	Ratchaburi Province, Thailand
Kaenpayorm1	Khon Kaen Province, Thailand

These accessions were grown in October 2010 at the Fruit Orchard Research Farm, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand. The accessions were arranged in a randomized complete block design with 3 replications. The spacing was 6 m between plants within row and 2 m between rows. N-P-K fertilizers formula 15–15–15 were applied at 15, 45 and 90 days after transplanting. Pests and diseases were controlled by weekly applications of insecticides and fungicides. Mini-sprinkler irrigation was available as necessary to avoid drought stress. Metal supports were also constructed for each plot.

The plants that produced male flowers were used as pollinators to pollinate all accessions in the experiment. The receptive flowers were artificially pollinated and the pollinated flowers were tagged and labeled.

Data collection

Data were recorded for fruit fresh weight, aril weight, days to fruit maturity, number of seeds, total carotenoids, lycopene, beta-carotene and lycopene per fruit. The fruits were harvested at ripening maturity which was determined by red skin of the fruits. Days to ripening was determined by numbers of days from artificial pollination to ripening maturity. Aril was separated from the fruits, put in sealable plastic bags and weight. Seed number per fruit was determined by counting filled seeds.

Sample preparation for phytochemical extraction

One fruit was randomly chosen from 3 ripening fruits for each of 3 sampling dates which were considered as 3 replications in unreplicated plot. The ripening fruits were determined by red fruits and red aril. The fruits were cleaned and separated into pulp, peeled seeds and aril. The aril samples were stored in a freezer (-20 ^{o}C) until further analysis.

Carotenoid extraction

Aril samples of 1 g each were oven-dried at 50 ^{o}C for 72 hours, and the dry samples were ground into powder using a mortar and a pestle. The powdered sample (0.1 g) was mixed with 50 ml of hexane : acetone : ethanol (2 : 1 : 1). Water of 5 ml was added into the mixed sample 2 times and the amount of water was 10 ml in total. The supernatant was placed in a round-bottomed flask and evaporated to dryness. The solid residue was re-dissolved in 8 ml of petroleum ether. The final solution of 2 ml was filtered through 0.45 µm membrane filter before injection of 20 µl into the high performance liquid chromatography (HPLC) aperture. Lycopene and beta-carotene were analyzed in triplicate using the HPLC by a method modified from Kubola and Siriamornpun (2011). Analysis was performed using Shimadzu LC-20AD pumps, a SPD-20M diode array detector, and inertsil ODS-3 C-18 column reverse phase (4.6 x 250 mm i.d., 5 µM). The mobile phase consisted of Acetonitrile methanol (solvent A)/ Dichloromethane (solvent B) and Methanol (solvent C) at a flow rate of 1 ml/min. The absorbance was read at 450 nm.

Statistical analysis

The data for the agronomic traits and phytochemical contents were analyzed statistically according to a randomized complete block design. Mean, minimum (min), maximum (max), standard error (SE), coefficient of variation (CV) and F-ratio were determined using Statistix-8 software. Least significant difference (LSD) at the 5% level was used to compare mean differences. The relationships among traits were calculated by the Pearson's correlation analysis using accession means (Ireland, 2010).

Cluster analysis

A data matrix of the 26 accessions was constructed using means for agricultural traits and phytochemical contents. Hierarchical agglomerative clustering was then performed for agricultural traits and phytochemical contents, using the Ward criterion. All calculations were performed using JMP Pro software (version 10.0, SAS institute Inc., Chicago, IL, USA).

RESULTS

Variations in spiny bitter gourd accessions

Variations in 26 accessions of spiny bitter gourd were evaluated for total carotenoids, lycopene, beta-carotene and lycopene per fruit, fruit fresh weight, aril weight, days to fruit maturity and number of seeds (Table 2). High variations were observed for all characters under investigation as indicated by high CV values (5 for days to maturity to 37 for lycopene per fruit) and high F-ratio (9.8 for total carotenoids to 152.7 for fruit fresh weight). The ranges of minimum and maximum values for these characters also indicated variations for these characters. Mean values for these characters are also provided for comparison with other investigations.

It is interesting to note here that some characters such as total carotenoids, lycopene content and lycopene yield per fruit had high CV values (22 - 37), but they had low F-ratios (9.8 - 20.4). In contrast to the above traits, fruit fresh weight, days to fruit maturity and number of seeds had low CV values (5 - 8), but they had high F-ratios (22.0 - 152.7).

Variation in fruit shape was also observed in these spiny bitter gourd accessions (Figure 1). There were 3 different fruit shapes including round (KKU ac. 09-008), elliptical with pointed end (KKU ac.09-002, KKU ac.09-016, KKU ac.09-010, KKU ac.09-003, KKU ac.10-049, Kaenpayorm1, KKU ac.09-018, KKU ac.09-027, KKU ac.09-030, KKU ac.10-090, KKU ac.09-019, KKU ac.10-077, KKU ac.09-004, KKU ac.09-034, KKU ac.09-012, KKU ac.09-033, KKU ac.10-087, KKU ac.10-080, KKU ac.09-011, KKU ac.09-013, KKU ac.09-020, KKU ac.10-043, KKU ac.10-086 and KKU ac.11-138) and flattened (oblate) (KKU ac. 10-094).

KKU ac. 10-094 had the highest fruit fresh weight (1787 g $fruit^{-1}$), aril weight (393 g $fruit^{-1}$) and days to fruit maturity (108 days after flowering), whereas KKU ac. 09-010 had the highest number of seeds (53 seed $fruit^{-1}$). Other accessions had smaller fruits and lower aril weight and matured earlier than KKU ac. 10-094 (Table 3). Also KKU ac. 10-094 had the highest total carotenoids (4041 μg g^{-1} dry weight), beta-carotene (1513 μg g^{-1} dry weight) and lycopene per fruit (52608 μg $fruit^{-1}$) and its lycopene content was also high although it was lower than 2487 μg g^{-1} dry weight of KKU ac. 09-008. Other interesting accessions included KKU ac. 09-002 because of high total carotenoids, KKU ac. 09-008 because of high total carotenoids and lycopene and KKU ac. 09-016 because of high total carotenoids (Table 4).

Correlations among quantitative characters

Positive and high correlation coefficients between total carotenoids with lycopene ($P \leq 0.01$, $r = 0.83$), beta-carotene ($P \leq 0.01$, $r = 0.74$) and lycopene per fruit ($P \leq 0.01$, $r = 0.73$) were observed. Total carotenoids also had positive correlations with days to fruit maturity ($P \leq 0.05$, $r = 0.46$), aril weight ($P \leq 0.05$, $r = 0.43$) and fruit fresh weight ($P \geq 0.05$, $r = 0.37$) although it was not significant, but it is not associated with number of seeds ($P \geq 0.05$, $r = 0.08$).

Lycopene was positively and significantly associated with beta-carotene ($P \leq 0.05$, $r = 0.48$) and lycopene per fruit ($P \leq 0.01$, $r = 0.62$), but it is not significantly associated with fruit fresh weight, aril weight, days to fruit maturity and number of seeds. Beta-carotene was associated with lycopene per fruit ($P \leq 0.01$, $r = 0.75$), fruit fresh weight ($P \leq 0.01$, $r = 0.59$), aril weight ($P \leq 0.01$, $r = 0.62$) and days to fruit maturity ($P \leq 0.01$, $r = 0.62$), but it is not associated with number of seeds. Similarly, lycopene per fruit was also associated with fruit fresh weight ($P \leq 0.01$, $r = 0.84$), aril weight ($P \leq 0.01$, $r = 0.90$) and days to fruit maturity ($P \leq 0.01$, $r = 0.86$), but it is not associated with number of seeds.

Fruit characters (fruit fresh weight and aril weight) and days to fruit maturity were inter-related with correlation coefficients ranging from $P \leq 0.01$, $r = 0.80$ to $P \leq 0.01$, $r = 0.94$, and fruit fresh weight was also associated with number of seeds ($P \leq 0.05$, $r = 0.44$). However, aril weight and days to fruit maturity were not associated with number of seeds ($P \geq 0.05$, $r = 0.27$) (Table 5).

Table 2. Means, standard errors (SE), minimum (min), maximum (max), coefficient of variation (CV) and F-ratio of quantitative characters of 26 accessions of spiny bitter gourd.

Characters	Mean ± SE	Min - Max	C.V. (%)	F-ratio
Total carotenoid (µg g^{-1} dry weight)	2013 ± 261	840-4041	23	9.8^{**}
Lycopene (µg g^{-1} dry weight)	1051 ± 131	364-2487	22	16.0^{**}
Beta-carotene (µg g^{-1} dry weight)	417 ± 51	100-1513	21	39.6^{**}
Lycopene per fruit (µg $fruit^{-1}$)	9746 ± 2099	2500-52608	37	20.4^{**}
Fruit fresh weight (g $fruit^{-1}$)	578 ± 23	280-1787	7	152.7^{**}
Aril weight (g $fruit^{-1}$)	87 ± 11	48-393	21	36.0^{**}
Days to fruit maturity (days)	60 ± 2	52-108	5	37.8^{**}
Number of seed (seed $fruit^{-1}$)	36 ± 2	21-53	8	22.0^{**}

** significant at $P < 0.01$

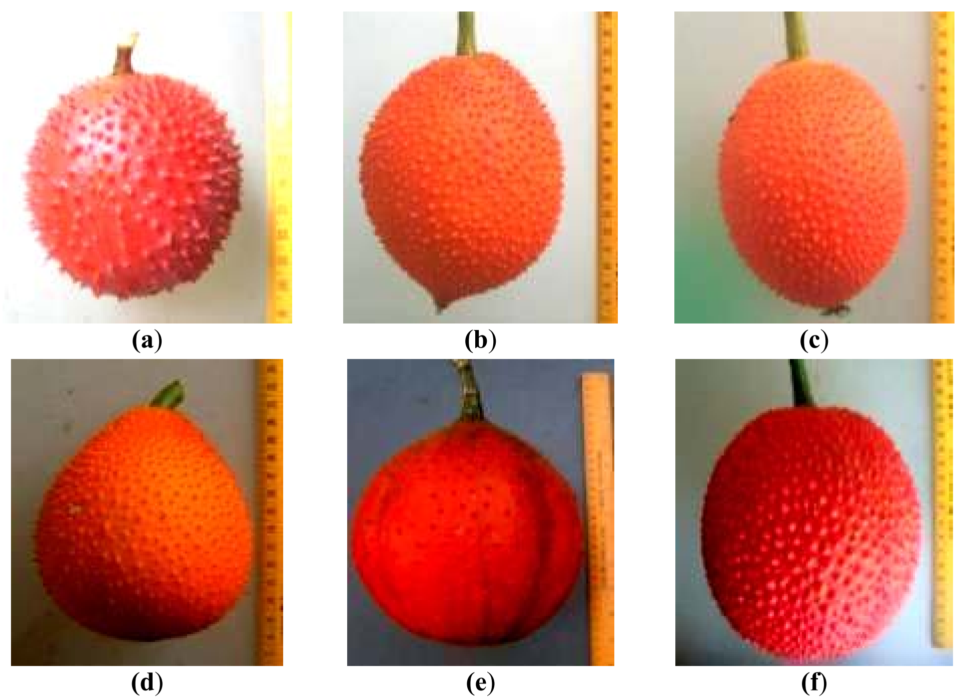

Figure 1. Genetic diversity of fruit types: (a) round; (b) elliptical with pointed end; (c) oval; (d) flattened bottom; (e) flattened (oblate); and (f) semi-cylindrical of *Momordica cochinchinensis* (Lour.) Spreng.

Table 3. Fruit fresh weight (g fruit^{-1}), aril weight (g fruit^{-1}), days to fruit maturity (days) and number of seeds (seeds fruit^{-1}) of 26 spiny bitter gourd accessions.

Accessions	Fruit fresh weight	Aril weight	Days to fruit maturity	Number of seeds
KKU ac.09-002	355 i	65 def	56 f-i	35 g-k
KKU ac.09-003	450 gh	65 def	63 c	23 n
KKU ac.09-004	725 c	110 bc	59 c-h	39 d-g
KKU ac.09-008	280 j	48 f	60 c-f	34 i-l
KKU ac.09-010	715 c	90 bcd	59 c-h	53 a
KKU ac.09-011	410 hi	60 def	58 d-h	34 h-l
KKU ac.09-012	460 gh	73 def	56 f-i	35 g-k
KKU ac.09-013	548 ef	80 cde	55 hi	30 lm
KKU ac.09-016	643 d	80 cde	55 ghi	42 def
KKU ac.09-018	455 gh	85 cde	58 d-h	36 g-k
KKU ac.10-019	478 g	85 cde	57 d-i	22 n
KKU ac.10-020	350 i	58 ef	52 i	21 n
KKU ac.09-027	495 fg	55 ef	59 c-h	28 m
KKU ac.09-030	553 ef	80 cde	58 d-h	37 f-j
KKU ac.09-033	715 c	82 cde	60 c-f	50 ab
KKU ac.09-034	673 cd	72 def	61 cde	33 j-m
KKU ac.10-043	568 e	67 def	60 c-g	44 cd
KKU ac.10-049	365 i	55 ef	62 cd	38 f-i
KKU ac.10-077	503 fg	65 def	55 ghi	37 f-j
KKU ac.10-080	555 ef	62 def	57 e-i	39 e-h
KKU ac.10-086	507 efg	85 cde	57 e-i	35 j-k
KKU ac.10-087	854 b	117 b	55 f-i	48 bc
KKU ac.10-090	645 d	88 bcd	54 hi	48 bc
KKU ac.10-094	1787 a	393 a	108 a	43 de
KKU ac.11-138	445 gh	63 def	59 c-h	31 klm
Kaenpayorm1	503 fg	83 cde	73 b	41 def
Mean	578	87	60	36

Mean in the same column followed with the same letter are not significant at $P < 0.05$ by LSD.

Table 4. Total carotenoids (µg g^{-1} dry weight), lycopene (µg g^{-1} dry weight), beta-carotene (µg g^{-1} dry weight), and lycopene per fruit (µg $fruit^{-1}$) of 26 spiny bitter gourd accessions.

Accessions	Total carotenoid	Lycopene	Beta-carotene	Lycopene per fruit
KKU ac.09-002	3551 a	1274 de	1159 b	13374 b-e
KKU ac.09-003	2599 b	1207 d-g	441 def	7169 f-j
KKU ac.09-004	1443 e-j	835 g-j	219 i-l	9764 c-g
KKU ac.09-008	3412 a	2487 a	141 kl	16024 b
KKU ac.09-010	2226 bcd	1513 cd	384 d-h	14545 bc
KKU ac.09-011	1934 b-g	827 hij	234 i-l	5033 g-j
KKU ac.09-012	1669 d-h	1294 de	358 d-i	9673 c-h
KKU ac.09-013	1550 d-j	555 i-m	242 h-l	4853 g-j
KKU ac.09-016	3447 a	1734 bc	763 c	14316 bcd
KKU ac.09-018	1830 c-h	789 h-k	248 g-k	7475 e-j
KKU ac.10-019	1910 b-g	808 hij	272 g-k	6864 f-j
KKU ac.10-020	925 ij	426 klm	100 l	2846 ij
KKU ac.09-027	2037 b-f	1272 def	715 b	7445 e-j
KKU ac.09-030	1572 d-j	805 hij	302 f-j	6776 f-j
KKU ac.09-033	1321 f-j	979 e-h	326 e-i	8560 c-i
KKU ac.09-034	2241 bcd	1515 cd	642 c	9474 d-h
KKU ac.10-043	1152 hij	364 m	235 i-l	2500 j
KKU ac.10-049	1572 d-j	946 e-h	484 d	6137 f-j
KKU ac.10-077	2150 b-e	900 f-i	382 d-h	6225 f-j
KKU ac.10-080	2465 bc	1791 bc	454 de	11449 b-f
KKU ac.10-086	840 j	400 lm	145 kl	3797 hij
KKU ac.10-087	1750 c-h	750 h-l	173 jkl	10246 b-g
KKU ac.10-090	1768 c-h	653 h-m	360 d-i	6158 f-j
KKU ac.10-094	4041 a	1893 b	1513 a	52608 a
KKU ac.11-138	1285 g-j	490 j-m	392 d-g	3269 ij
Kaenpayorm1	1654 d-i	826 hij	163 jkl	6823 f-j
Mean	2013	1051	417	9746

Means in the same column followed with the same letter are not significant at $p < 0.05$ by LSD.

Table 5. Correlation coefficients among quantitative characters of 26 accessions of spiny bitter gourd.

Characters	Total carotenoid	Lycopene	Beta-carotene	Lycopene per fruit	Fruit fresh weight	Aril weight	Days to fruit maturity
Lycopene	0.83**						
Beta-carotene	0.74**	0.48*					
Lycopene per fruit	0.73**	0.62**	0.75**				
Fruit fresh weight	0.37^{ns}	0.24^{ns}	0.59**	0.84**			
Aril weight	0.43*	0.24^{ns}	0.62**	0.90**	0.94**		
Days to fruit maturity	0.46*	0.34^{ns}	0.62**	0.86**	0.80**	0.89**	
Number of seeds	0.08^{ns}	0.13^{ns}	0.09^{ns}	0.27^{ns}	0.44*	0.27^{ns}	0.18^{ns}

$^{ns, *, **}$ non-significant and significant at 0.05 and 0.01 probability levels, respectively (n = 26)

Cluster analysis

A dendrogram constructed based on agronomic traits and phytochemical contents grouped 26 accessions of spiny bitter gourd into 6 distinct clusters (Figure 2), and the characters of each group were summarized in Table 6.

Cluster A consisted of KKU ac.09-002, KKU ac.09-010, KKU ac.09-016 and KKU ac.10-080. This cluster had total carotenoids between 2226 to 3551 µg g^{-1} dry weight, lycopene between 1274 to 1791 µg g^{-1} dry weight, beta-carotene between 384 to 1159 µg g^{-1} dry weight, lycopene per fruit between 11449 to 14545 µg $fruit^{-1}$, fruit fresh weight between 355 to 715 g $fruit^{-1}$, aril weight between 65 to 90 g $fruit^{-1}$, days to fruit maturity between 55 to 59 days after flowering, number of seeds between 35 to 53 seeds $fruit^{-1}$ and elliptical with pointed end fruit type.

Cluster B had 1 accession (KKU ac. 09-008). This accession had 3412 µg g^{-1} dry weight of total carotenoids, 2487 µg g^{-1} dry weight of lycopene, 141 µg g^{-1} dry weight of beta-carotene, 16024 µg $fruit^{-1}$ of lycopene per fruit, 280 g $fruit^{-1}$ of fresh fruit weight, 48 g $fruit^{-1}$ of aril weight, 60 days after flowering of fruit maturity, 34 seeds $fruit^{-1}$ and round fruit type.

Cluster C comprised KKU ac.09-003, KKU ac.09-019, KKU ac.09-027, KKU ac.09-034, KKU ac.09-011, KKU ac.09-018, KKU ac.09-030, KKU ac.10-077, KKU ac.09-012 and KKU ac.10-049. This cluster had total carotenoids between 1572 to 2599 µg g^{-1} dry weight, lycopene between 789 to 1515 µg g^{-1} dry weight, beta-carotene between 234 to 715 µg g^{-1} dry weight, lycopene per fruit between 5033 to 9673 µg $fruit^{-1}$, fruit fresh weight between 365 to 673 g $fruit^{-1}$, aril weight between 55 to 85 g $fruit^{-1}$, days to fruit maturity between 55 to 63 days after flowering, number of seeds between 22 to 38 seeds $fruit^{-1}$ and elliptical with pointed end fruit type.

Cluster D consisted of KKU ac.09-013, KKU ac.11-138, KKU ac.10-086 and KKU ac.10-020. This cluster had total carotenoids between 840 to 1550 µg g^{-1} dry weight, lycopene between 400 to 555 µg g^{-1} dry weight, beta-carotene between 100 to 392 µg g^{-1} dry weight, lycopene per fruit between 2846 to 4853 µg $fruit^{-1}$, fruit fresh weight between 350 to 555 g $fruit^{-1}$, aril weight between 58 to 80 g $fruit^{-1}$, days to fruit maturity between 52 to 59 days after flowering, number of seeds between 21 to 39 seeds $fruit^{-1}$ and elliptical with pointed end fruit type.

Cluster E consisted of KKU ac.09-004, KKU ac.09-033, KKU ac.10-090, KKU ac.10-087, KKU ac.10-043 and Kaenpayorm 1. This cluster had total carotenoids between 1552 to 1768 µg g^{-1} dry weight, lycopene between 364 to 979 µg g^{-1} dry weight, beta-carotene between 163 to 360 µg g^{-1} dry weight, lycopene per fruit between 2500 to 10246 µg $fruit^{-1}$, fruit fresh weight between 503 to 854 g $fruit^{-1}$, aril weight between 67 to 117 g $fruit^{-1}$, days to fruit maturity between 54 to 73 days after flowering, number of seeds between 39 to 50 seeds/fruit and elliptical with pointed end fruit type.

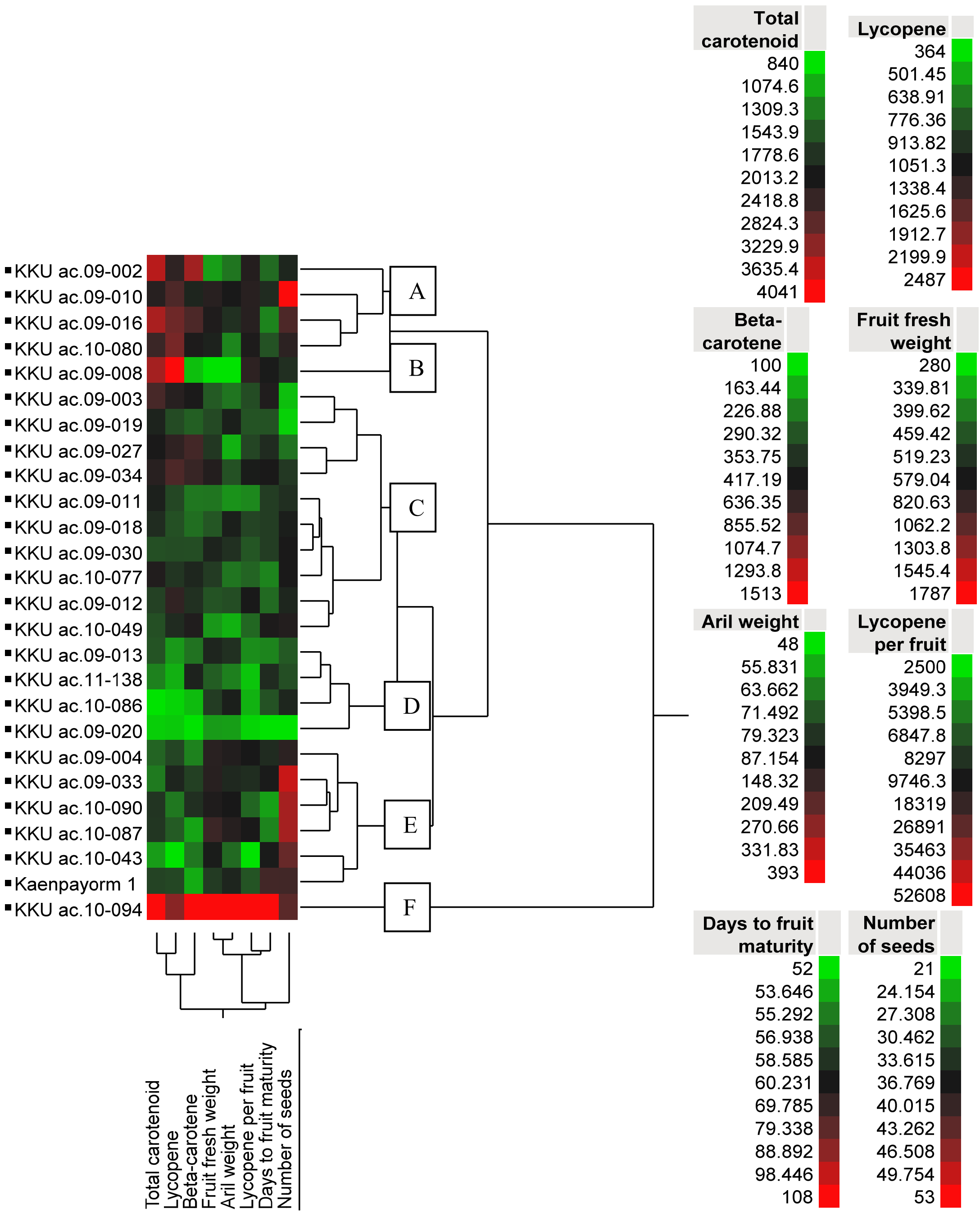

Figure 2. Dendrogram showing genetic relatedness of 26 spiny bitter gourd accessions collected in Thailand and Vietnam. Six main clusters (A to F) were formed. Two-way clustering method was Ward's cluster analysis based on agronomic traits and phytochemicals content (scale: Distance scale)[a].

[a] Cluster together columns as well as rows; the columns must be measured on the same scale.

Table 6. List of sites of collection, total carotenoids (μg g^{-1} dry weight), lycopene (μg g^{-1} dry weight), beta-carotene (μg g^{-1} dry weight), lycopene per fruit (μg $fruit^{-1}$), fruit fresh weight (g $fruit^{-1}$), aril weight (g $fruit^{-1}$), days to fruit maturity (days) and number of seeds (seeds $fruit^{-1}$) in 7 clusters grouped by cluster analysis of 26 spiny bitter gourd accessions.

Groups	Collection in site	Total carotenoids	Lycopene	Beta-carotene	Lycopene per fruit	Fruit fresh weight	Aril weight	Days to fruit maturity	Number of seeds per fruit
1	Khon Kaen (2), Nakhon Pathom (1), Hybrid (1)	2226-3551	1274-1791	384-1159	11449-14545	355-715	65-90	55-59	35-53
2	Chiang Mai (1)	3412	2487	141	16024	280	48	60	34
3	Khon Kaen (2), Yasothon (1), Kalasin (3), Nong Khai (1), Chaiyaphum (1), Hybrid (2)	1572-2599	789-1515	234-715	5033-9673	365-673	55-85	55-63	22-38
4	Kalasin (1), Ratchaburi (1), Hybird (2)	840-1550	400-555	100-392	2846-4853	350-555	55-80	52-59	21-39
5	Samut Songkhram (1), Hybrid (1), Khon Kaen (2), Nakhon Pathom (1), Kanchanaburi (1)	1152-1768	364-979	163-360	2500-10246	503-854	67-117	54-73	39-50
6	Vietnam (1)	4041	1893	1513	52608	1787	393	108	43

The number in parenthesis is the number of accessions.

Cluster F had 1 accession (KKU ac. 10-094) collected from Vietnam. This accession had 4041 µg/g dry weight of total carotenoid, 1893 µg g^{-1} dry weight of lycopene, 1513 µg g^{-1} dry weight of beta-carotene, 52608 µg $fruit^{-1}$ of lycopene per fruit, 1787 g $fruit^{-1}$ of fresh fruit weight, 393 g $fruit^{-1}$ of aril weight, 108 days after flowering to fruit maturity, 43 seeds $fruit^{-1}$ and flattened (oblate) fruit type.

DISCUSSION

Variations in spiny bitter gourd accessions

Spiny bitter gourd is an underutilized vegetable crop in Southeast Asia. People in this region use spiny bitter gourd as coloration in many food recipes such as sticky rice, several sweet recipes and soft drinks. As spiny bitter gourd has very high lycopene and beta-carotene, it has high potential for development as a functional food. This study used fruit characters (fruit fresh weight, aril weight, number of seeds and fruit maturity) and phytochemical characters (lycopene content, beta-carotene content and total carotenoids) to evaluate genetic variability in spiny bitter gourd. This information is limited because the crop is rather new for breeders and horticulturists. In Thailand, the fruit of spiny gourd is usually collected from fence climbers or from wild plants. It is found seasonally in local markets (June to November). Statistical data are lacking. There is very little information on genetic variations, and no cultivar selection has been reported. Germplasm collections are made by villagers with home gardens.

Based on CV values and F-ratios, high variations were observed in 26 accessions of spiny bitter gourd collected in Thailand and Vietnam for fruit fresh weight, aril weight, number of seeds, days to fruit maturity, lycopene content, beta-carotene content and total carotenoids. The results are convincing to use this germplasm collection for breeding purpose.

Scant information on variations in these characters is available in the literature. During literature review, we could not find report on variations in fruit characters and fruit maturity. However, total carotenoids concentration on average was 497 µg g^{-1} fresh material with lycopene dominating and exceeding beta-carotene concentrations by a factor of approximately 5 (408 µg g^{-1} versus 83 µg g^{-1}) (Vuong *et al.,* 2006). Lycopene was found to be predominantly present in the gac seed membrane at a concentration of up to 380 µg g^{-1} of seed membrane (Aoki *et al*., 2002). In another study, mean values obtained in aril tissues were 1342 µg trans, 204 µg cis, and 2227 µg total lycopene; 597 µg trans, 39 µg cis and 718 µg total beta-carotene (Ishida *et al.,* 2004). In comparison of 3 studies, carotenoid concentration, lycopene concentration and beta-carotene concentration in this study (2013 µg g^{-1} total carotenoids, 1051 µg g^{-1} lycopene and 417 µg g^{-1} total beta-carotene) were much higher than those reported by Vuong *et al.* (2006) and Aoki *et al.* (2002) because the results in this study were based on dry weight, but the results in this study seemed to be lower than those reported by Ishida *et al.* (2004). Carotenoid levels in spiny bitter gourd seem to vary widely.

High F-ratio indicated that genotype contributed to large portion of total variation for fruit fresh weight, beta-carotene, days to fruit maturity, aril weight, number of seeds, lycopene per fruit, lycopene and total carotenoid, and improvement of these traits will be easy. In this study, spiny bitter gourd accessions with good fruit characters and high phytochemical characters were identified, and these accessions will be further used in breeding programs.

Correlations among quantitative characters

Correlations among characters are important when breeding for multiple characters is carried out, and selection for one trait may affect other traits. In this study, total carotenoids, lycopene, beta-carotene and lycopene per fruit were inter-related with correlation coefficients ranging from 0.48 between lycopene and beta-carotene to 0.83 between total carotenoids and lycopene. The results also indicated that fruit characters were also interrelated with correlation coefficients ranging from 0.80 between fruit fresh weight and days to maturity and 0.94 between aril weight and fruit fresh weight. It is also interesting to note here that all phytochemicals were also associated with fruit characters as beta-carotene and lycopene per fruit had strong associations with fruit fresh weight, aril weight and days to fruit maturity, whereas the relationships between total carotenoids and lycopene with fruit fresh weight, aril weight and days to fruit maturity were rather weak. Most characters under investigation were not associated with number of seeds except for fruit fresh weight.

In previous study, inverse correlation between beta-carotene and lycopene concentrations was observed in spiny bitter gourd due to the ripening stage, since lycopene appears earlier than beta-carotene in the carotenogenesis pathway, and less mature fruits contain higher lycopene and lower beta-carotene concentrations than more mature fruits (Vuong *et al.,* 2006).

The results indicated that improvement of fruit characters and phytochemicals at the same time would be possible especially for beta carotene and lycopene per fruit, but it might be difficult to improve total carotenoids and lycopene at the same time with improvement of fruit fresh weight, aril weight and days to fruit maturity because of weak correlations. Improvement of fruit fresh weight would result in increase in seed number. However, high seed number is not necessary for this crop because the crop can be propagated vegetatively, but seeds are still important for breeding purpose.

Cluster analysis

A dendrogram from cluster analysis based on fruit characters and phytochemical characters divided 26 spiny bitter gourd accessions into 6 distinct clusters. The accession from Vietnam (KKU ac. 10-094) formed cluster F which was isolated from other 5 clusters which were the accessions collected in Thailand. KKU ac. 10-094 also had flattened (oblate) fruit shape, whereas other accessions in Thailand had round fruits (cluster B) (KKU ac. 09-008) and elliptical with pointed end fruits (clusters A, C, D and E). Actually, 6 fruit shapes were found in our germplasm collections including round, flattened, elliptical with pointed end, oval, flattened bottom and semi-cylindrical (Figure 1).

It is very interesting to note here that an accession from Vietnam was the out-group of these accessions and it also had many good characters. As spiny bitter gourd is grown and utilized mostly in Vietnam, germplasm in Vietnam is valuable for immediate use and further development. A germplasm collection mission was first carried out in central Vietnam during March 2012, and many accessions were collected for further evaluation and utilization. The results in this study revealed genetic difference of the spiny bitter gourd germplasm in these countries.

In our previous findings in 25 accessions of spiny bitter gourd common to this study, 11 RAPD primers could divide these accessions into 8 clusters (Bootprom *et al.,* 2012). In comparison of the 2 studies, both dendrograms separated the accession KKU ac. 10-094 from Vietnam from other accessions. The clustering pattern based on RAPD markers was not in accordance with the grouping based on fruit and phytochemical characters. The lack of relationships between molecular study and conventional study for grouping of germplasm has also been reported in bitter gourd (*Momordica charantia*) (Paul and Raychaudhuri, 2010). Similarly, in *Cuminum cyminum*, a valuable medicinal plant belonging to family Apiaceae, grouping based on molecular markers and morpho-agronomic traits are different so these 2 systems could not discriminate accessions as in the same way (Rostami-Ahmadvandi *et al.,* 2013).

The results also indicated that agronomic traits and phytochemical contents are useful parameters for evaluation of genetic diversity in spiny bitter gourd. Although newer methods of genetic diversity evaluation in crop plants have been invented and used widely, the conventional methods are still useful and more cost effective.

For development of spiny bitter gourd as functional food and natural food colorant in food industry, more breeding attempts are still required. The breeding activities include germplasm collection, preliminary evaluation of new germplasm and dialell analysis of crosses among accessions for agricultural traits and phytochemical contents to understand heterotic patterns of spiny bitter gourd germplasm and to find appropriate ways for germplasm utilization. The results will enable breeders to make informed decisions about possible heterotic groups for their breeding programs and germplasm conservation.

CONCLUSION

In this study, grouping of germplasm into different clusters using agricultural traits and level of phytochemical contents was a means to identify genetic differences of 26 spiny bitter gourd accessions. This knowledge will enable useful information for germplasm conservation, utilization and management for the development of spiny bitter gourd in breeding programs.

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