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GENETICS DIVERSITY BASED ON HORTICULTURAL TRAITS AND TOTAL SOLUBLE SOLID CONTENT IN MULBERRY (*Morus alba*) VARIETIES

N. BOOTPROM[1], P. SONGSRI[1]*, N. KONGPUN[2] and A. KAMTOU[1]

[1] Plant Breeding Research Center for Sustainable Agriculture, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Queen Sirikit Sericulture Center, Sisaket, Phen District, Srisaket Province, 33000, Thailand
*Corresponding author's email: patcharinso@kku.ac.th

SUMMARY

Mulberry (*Morus alba*) is a highly nutritious fruit as its contains high phytochemicals especially for anthocyanin. The information on genetic variation in germplasm is important for development of new varieties. The objective of this study was to evaluate genetic diversity of mulberry based on horticultural traits and total soluble solid content. Twenty-one varieties of mulberry collected from different parts of Thailand and Lao People's Democratic Republic were evaluated at the fruit orchard section, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University. Genetic diversity of 21 varieties of mulberry was identified base on horticultural traits (fruit weight, length and width) and total soluble solid at immature, medium ripe and fully ripe stages. High variations were observed in these varieties for all horticultural traits and total soluble solid. At the coefficient of determination (R^2) of 0.89, cluster analysis showed that the 21 varieties of mulberry could be grouped into 6 distinct clusters. The results are useful for germplasm conservation, utilization and management for breeding of mulberry in the future.

Keywords: Breeding, cluster analysis, mulberry, germplasm, total soluble solid content

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INTRODUCTION

Mulberry belongs to the Morus genus of the Moraceae family (Orban and Ercisli, 2010; Arfan *et al.*, 2012), and there are 24 species of Morus with at least 100 known varieties distributed in Asia, Africa, Europe, and North, Central and South America (Nepal and Ferguson, 2012). About 68 related species of mulberry have been found in the world and most of them are in Asia (Datta, 2002).

Morus species are economically important to the silk industry as they are used for rearing silkworm (Bombyx mori L.) larvae (Watanabe, 1958; Nepal and Ferguson, 2012). Mulberry leaves, bark and branches have long been used in Chinese medicine (Zhishen *et al.*, 1999; Arfan *et al.*, 2012). In most European countries mulberries are grown for fruit production (Gerasopoulos and Stavroulakis, 1997; Ercisli, 2004; Arfan *et al.*, 2012).

Plants of the genus Morus are known to be a rich source of flavonoids including quercetin 3-(-malonylglucoside), rutin, isoquercitin (Katsube *et al.*, 2006; Arfan *et al.*, 2012), cyanidin 3 rutinoside and cyanidin 3-glucoside (Chen *et al.*, 2006; Kang *et al.*, 2006; Arfan *et al.*, 2012). Ercisli and Emine (2007) reported a high content of total phenolics in white, red and black mulberry fruits. These properties are beneficial to health, and the fruit can be consumed in many ways from simple fresh fruit,

fruit juice, concentrated fruit juice and mulberry wine.

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 phytochemical content. Breeding for high and stable phytochemical content and yield is important for industrial utilization of mulberry, and the information on genetic diversity among germplasm is also important for effective breeding programs.

In Thailand, 46 accessions of mulberry were collected since 1985. Of these accessions, 37 accessions were local varieties that has long been cultivated in Thailand, 5 and 4 accessions were introduced from Japan and Europe, respectively (Silk research institute, 2002).

Although molecular techniques are available for evaluation of genetic diversity in crop plants, they are still very expensive for resource-limited projects and conventional evaluation using horticultural traits and total soluble solid is still useful. This information is not available for breeders to select parents for improvement of fruit mulberry. The objective of this study was to determine genetic diversity and genetic relatedness among 20 accessions of mulberry collected in Thailand and 1 variety collected in Lao People's Democratic Republic using horticultural traits and total soluble solid. The information will be of great benefit for genetic improvement of this crop.

MATERIALS AND METHODS

Plant materials

Twenty-one accessions of mulberry were evaluated in study. Twenty accessions were collected in Thailand and 1 accession was collected from Lao People's Democratic Republic (Table 1). These accessions were initially planted in 2007 at the fruit orchard section, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand at spacing of 2×2 m between plants.

The accession "Namprom" was used as a root stock, which was established previously at the orchard, and there were 6 plants for each accessions. Budding was carried out for each accession, and the new stems were cut at 60-80 cm from the ground or at the center of the stems in July 2008. The plants were further allowed regrowth for 2 months and then pruned, leaving 6-8 branches on the stems. At 8 months after budding, all leaves on the stems were removed to initiate fruit production.

NPK fertilizers formula 15-15-15 were monthly applied at 10 g/plant. Pests and diseases were controlled by weekly applications of insecticides and fungicides. Irrigation was supplied as and when necessary to avoid drought stress. Metal supports were also constructed for each plot.

Data collection

Five fruits were randomly chosen at immature (red), medium ripe (purple) and fully ripe (black) stages in each accessions. Data were recorded for fruit weight, fruit length, fruit width, and total soluble solid was recorded using refractometer.

Statistical analysis

The data for the horticultural traits and total soluble solid 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 (Statistix 8, 2007). 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 21accessions of mulberry was constructed using means for horticultural traits and total soluble solid. The cluster analysis based on Ward’s method and squared Euclidian distance was performed and the dendrogram was

Table 1. List of mulberry accessions used in this study and sites of collection.

Varieties	Collection sites
Pagsam 1	Chiang Mai Province, Thailand
Pagsam 2	Chiang Mai Province, Thailand
Pagsam 5	Chiang Mai Province, Thailand
Pagsam 6	Chiang Mai Province, Thailand
Pagsam 7	Chiang Mai Province, Thailand
Khunvang 1	Chiang Mai Province, Thailand
Khunvang 2	Chiang Mai Province, Thailand
Khunkang 3	Chiang Mai Province, Thailand
Khunkang 4	Chiang Mai Province, Thailand
Khunkang 5	Chiang Mai Province, Thailand
Chumpon	Udontani Province, Thailand
Pikultong	Udontani Province, Thailand
Srisaket hybrid	Udontani Province, Thailand
Changmai	Udontani Province, Thailand
Bansuanjaroun	Chiang Rai Province, Thailand
Bantamavang	Chiang Mai Province, Thailand
Banpankang	Chiang Mai Province, Thailand
Banmaesalongnai	Chiang Rai Province, Thailand
Maesalong 2	Chiang Rai Province, Thailand
Namprom	Chaiyaphum Province, Thailand
Ponsavan	Lao People's Democratic Republic

constructed. All calculations were performed in SAS 6.12 software (SAS, 2001).

RESULTS

Variations in mulberry accessions

Variations in 21varieties of mulberry were evaluated for fruit weight, fruit length, fruit width, total soluble solid at different maturity stages of fruits. High variations were observed for all characters under investigation as indicated by high CV values (7.9 for total soluble solid at fully ripe stage to 30.6 for fruit fresh weight) and high F-ratio (3.5 for total soluble solid at immature stage to 7.6 for fruit length) (Table 2). 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 fruit fresh weight had high CV values (30.6), but they had low F-ratios (4.7). In contrast to the above traits, fruit width had low CV values (9.3), but they had high F-ratios (7.3).

Fruit fresh weights ranging from 0.9 to 2.9 g were observed among mulberry accessions, and the accessions were also different for fruit length and fruit width, ranging from 18.4 to 32.3 mm and 9.0 to 13.5 mm, respectively. Total soluble solid increased with fruit maturity, and

Table 2. Means, standard errors (SE), minimum (min), maximum (max), coefficient of variation (CV) and F-ratio for fruit fresh weight, fruit length, fruit width and total soluble solid (TSS) of 21 mulberry accessions evaluated at immature, medium ripe and fully ripe stages.

Characters	Mean ± SE	Min- Max	C.V. (%)	F-ratio
Fruit weight (g)	1.6 ± 0.4	0.9-2.9	30.6	4.7**
Fruit length (mm)	24.1 ± 2.0	18.4-32.3	11.5	7.6**
Fruit width (mm)	10.6 ± 0.7	9.0-13.5	9.3	7.3**
TSS at immature stage (°Brix)	8.5 ± 0.6	7.2-10.0	9.1	3.5**
TSS at medium ripe stage (°Brix)	12.6 ± 0.8	10.6-16.0	9.3	4.8**
TSS at fully ripe stage (°Brix)	17.3 ± 1.0	13.6-19.5	7.9	5.4**

** significant at $P < 0.01$

this trait was low at immature stage, medium and intermediate stage and high at ripe stage. There were also variations in total soluble solid at all maturity stages. At immature stage, total soluble solid values ranged from 7.2 to 10.0 °Brix. The total soluble solid values were higher, ranging from 10.6 to 16.0 °Brix at intermediate stage and were highest, ranging from 13.6 to 19.5°Brix at ripe stage.

The accessions with good fruit characters and high total soluble solid were identified. Bansuanjaroun was the best accession for fruit fresh weight (2.9 g), fruit length (32.3 mm) and fruit width (13.5 mm), but it had low total soluble solid (Table 3). Pagsam 1 and Pagsam 6, for example, had the highest total soluble solid at ripe stage, but they did not have big fruits.

Correlations among quantitative characters

Fruit fresh weight, length and width were inter-related with correlation coefficients ranging from $P \leq 0.01$, r = 0.83 to $P \leq 0.01$, r = 0.90 (Table 4). Total soluble solid values at all fruit maturity stages in general were negatively related with fruit fresh weight, fruit length and fruit width as indicated by negative correlations ranging from -0.19 ($P \geq 0.05$) to -0.32 ($P \leq 0.01$). Total soluble solid values at all fruit maturity stages in general were inter-related, and the relationship was strongest between medium maturity stage and ripe maturity stage (r = 0.48; ($P \leq 0.01$).

Cluster analysis

A dendrogram constructed based on horticultural traits and total soluble solid grouped 21 accessions of mulberryinto 6 distinct clusters at the coefficient of determination (R^2) of 0.89 (Figure 1) and the characters of each group were summarized in Table 5. Cluster 1 consisted of Pagsam 1, Pagsam 2, Pagsam 5, Pagsam 6, Pagsam 7, Khunvang 2, Namprom and Ponsavan. This cluster had fruit weight between 1.2 to 1.3 g, fruit length between 20.0 to 21.8 mm, fruit width between 9.3 to 10.1mm, total soluble solid at immature stage between 7.6 to 10.0 °Brix, total soluble solid at medium ripe stage between 12.4 to 16.0°Brix and total soluble solid at fully ripe stage between 17.6 to 19.5 °Brix.

Cluster 2 consisted of Khunvang 1, Khunvang 4 and Changmai. This cluster had fruit weight between 1.3 to 1.4 g, fruit length between 21.9 to 22.8 mm, fruit width between 9.8 to 10.7 mm, total soluble solid at immature stage between 8.0 to 8.6 °Brix, total soluble solid at medium ripe stage between 10.8 to 12.3 °Brix and total soluble solid at fully ripe stage between 15.6 to 18.4 °Brix.

Cluster 3 had 1 variety (Khunvang 3). This variety had fruit weight 0.9 g, fruit length 18.4 mm, fruit width 9.0 mm, total soluble solid at immature stage 8.4 °Brix, total soluble solid at medium ripe stage 12.9 °Brix and total soluble solid at fully ripe stage 15.0 °Brix.

Table 3. Fruit weight, fruit length, fruit width, total soluble solid (TSS) at immature, medium ripe and fully ripe stages of 21 mulberry accessions.

Accessions	Fruit fresh weight (g)	Fruit length (mm)	Fruit width (mm)	TSS (°Brix)		
				Immature stage	Medium ripe stage	Fully ripe stage
Pagsam 1	1.2gh	21.1fg	9.6fgh	9.0a-d	14.4ab	19.5a
Pagsam 2	1.2gh	20.8fg	9.3fgh	9.9ab	12.6c-g	17.6a-e
Pagsam 5	1.2gh	21.6efg	9.3gh	9.5abc	12.9b-f	19.1ab
Pagsam 6	1.3gh	21.7efg	9.3gh	8.9b-e	12.4c-h	19.3a
Pagsam 7	1.3fgh	21.5efg	9.6fgh	10.0a	13.6bcd	18.6abc
Khunvang 1	1.3fgh	22.8def	9.8fgh	8.6c-f	12.3c-h	17.0c-g
Khunvang 2	1.2gh	21.8efg	10.1e-h	8.0d-g	16.0a	19.0ab
Khunkang 3	0.9h	18.4g	9.0h	8.4def	12.9b-f	15.0hi
Khunkang 4	1.4d-h	21.9efg	9.8fgh	8.4def	11.8e-i	18.4a-d
Khunkang 5	2.0b-f	25.1cde	11.3b-e	8.6c-f	11.3f-i	17.2b-f
Chumpon	2.0b-f	27.5bc	12.0bcd	8.0d-g	10.6i	13.6i
Pikultong	2.3abc	28.8abc	12.1bc	8.2d-g	12.5c-g	15.1ghi
Srisaket hybrid	1.4e-h	26.3cd	10.7d-g	8.4def	12.2d-i	17.3b-f
Changmai	1.4e-h	22.7def	10.7c-f	8.0d-g	10.8hi	15.6fgh
Bansuanjaroun	2.9a	32.3a	13.5a	7.7eg	12.6c-g	16.5d-h
Bantamavang	2.1bcd	27.1c	11.7bcd	8.2d-g	11.2ghi	17.3b-f
Banpankang	2.0b-e	26.6cd	11.7bcd	7.2g	11.4f-i	16.1e-h
Banmaesalongnai	1.8c-g	27.1c	12.4ab	7.9efg	13.8bcd	17.3b-f
Maesalong 2	2.5ab	31.1ab	12.4ab	8.5c-f	12.3c-h	17.4b-f
Namprom	1.3gh	20.9fg	10.0e-h	9.0a-d	13.4b-e	18.5abc
Ponsavan	1.3gh	20.0fg	9.3fgh	7.6fg	13.9bc	18.9abc
Mean	1.6	24.1	10.6	8.5	12.6	17.3

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

Table 4. Correlation coefficients among fruit fresh weight, fruit length, fruit width and total soluble solid (TSS) of 21 mulberry accessions evaluated at immature, medium ripe and fully ripe stages.

Characters	Fruit fresh weight	Fruit length	Fruit width	TSS	
				Immature stage	Medium ripe stage
Fruit length	0.87**				
Fruit width	0.83**	0.90**			
TSS at immature stage	-0.23*	-0.26*	-0.28**		
TSS at medium ripe stage	-0.27*	-0.32**	-0.27*	0.22*	
TSS at fully ripe stage	-0.19ns	-0.24*	-0.27*	0.12ns	0.48**

ns, *,** non significant and significant at 0.05 and 0.01 probability levels, respectively (n = 21)

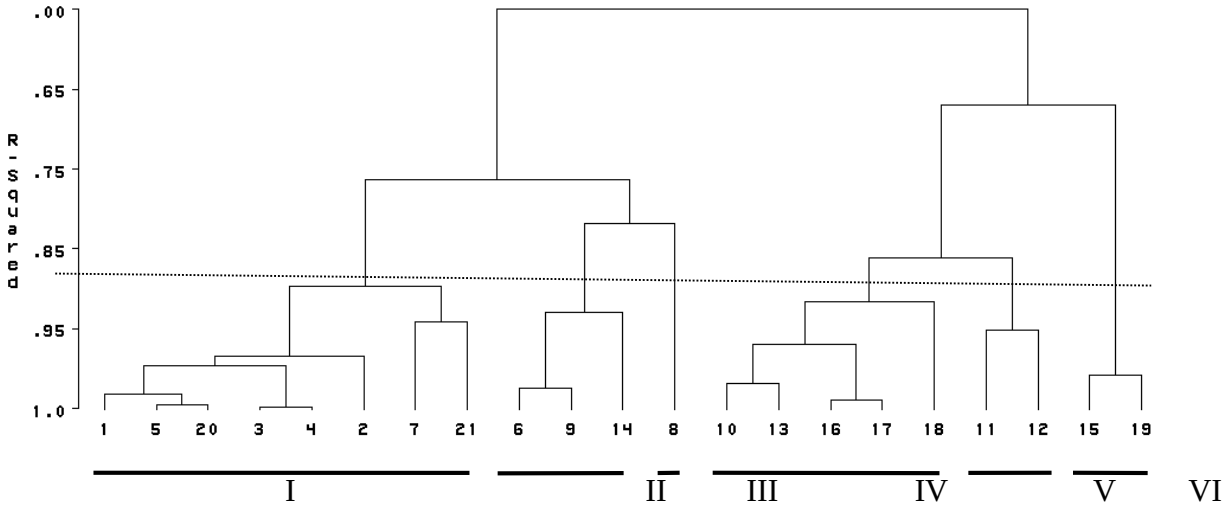

Figure 1. Dendrogram shows genetic relatedness of 21 mulberry accessions based on horticultural traits and total soluble solid.

1 = Pagsam 1; 2 = Pagsam 2; 3 = Pagsam 5; 4 = Pagsam 6; 5 = Pagsam 7; 6 = Khunvang 1; 7 = Khunvang 2; 8 = Khunvang 3; 9 = Khunvang 4; 10 = Khunvang 5; 11 = Chumpon; 12 = Pikultong; 13 = Srisaket hybrid; 14 = Changmai; 15 = Bansuanjaroun; 16 = Bantamavang; 17 = Banpankang; 18 = Banmaesalongnai; 19 = Maesalong 2; 20 = Namprom and21 = Ponsavan.

Table 5. Groups of mulberry accessions based on fruit weight, fruit length, fruit width, total soluble solid (TSS) at immature, medium ripe and fully ripe stages and their sites of collection.

Groups	Collection site	fruit weight (g)	fruit length (mm)	fruit width (mm)	TSS (°Brix)		
					Immature stage	Medium ripe stage	Fully ripe stage
1	Chiang Mai (6), Chaiyaphum (1), Laos (1)	1.2-1.3	20.0-21.8	9.3-10.1	7.6-10.0	12.4-16.0	17.6-19.5
2	Chiang Mai (2), Udontani (1)	1.3-1.4	21.9-22.8	9.8-10.7	8.0- 8.6	10.8-12.3	15.6-18.4
3	Chiang Mai (1)	0.9	18.4	9.0	8.4	12.9	15.0
4	Chiang Mai (3), Udontani (1), Chiang Rai (1)	1.4-2.1	25.1-27.1	10.7-11.7	7.2-8.6	11.2-12.2	16.1-17.3
5	Udontani (2)	2.0-2.3	27.5-28.8	12.0-12.1	8.0-8.2	10.6-12.5	13.6-15.1
6	Chiang Rai (2)	1.8-2.5	27.1-31.1	12.4-12.4	7.9-8.5	13.8-12.3	17.3-17.4

The number in parenthesis is the number of accessions.

Cluster 4 consisted of Khunvang 5, Srisaket hybrid, Bantamavang, Banpankang and Banmaesalongnai. This cluster had fruit weight between 1.4 to 2.1 g, fruit length between 25.1 to 27.1 mm, fruit width between 10.7 to 11.7 mm, total soluble solid at immature stage between 7.2 to 8.6 °Brix, total soluble solid at medium ripe stage between 11.2 to 12.2 °Brix and total soluble solid at fully ripe stage between 16.1 to 17.3 °Brix.

Cluster 5 had 2 varieties (Chumpon and Pikultong). This cluster had fruit weight 2.0 and 2.3 g respectively, fruit length 27.5 and 28.8 mm, respectively, fruit width 12.0 and 12.1 mm, respectively, total soluble solid at immature stage 8.0 and 8.2 °Brix respectively, total soluble solid at medium ripe stage 10.6 and 12.5 °Brix respectively and total soluble solid in fully ripe stage 13.6 and 15.1 °Brix, respectively.

Cluster 6 had 2 varieties (Bansuanjaroun and Maesalong2). This cluster had fruit weight 1.8 and 2.5 g, respectively, fruit length 27.1 and 31.1 mm, respectively, fruit width 12.4 and 12.4 mm, respectively, total soluble solid at immature stage 7.9 and 8.5 °Brix, respectively, total soluble solid at medium ripe stage 13.8 and 12.3 °Brix, respectively and total soluble solid at fully ripe stage 17.3 and 17.4 °Brix, respectively.

DISCUSSION

Variations in mulberry varieties

Mulberry is well recognized as a crop for silkworm rearing, but it is considered as an underutilized fruit crop. As mulberry has very high anthocyanin, it has high potential for development as functional food. Currently, mulberry fruits are used in many food recipes such as yam, wine, several sweet recipes and soft drinks.

There is very little information on genetic variations, and very little attempt, if any, has been made for crop breeding. In Thailand, fruit of mulberry is usually collected from wild plants, and statistical data for crop utilization is lacking. Germplasm collections are made by villagers and they grow the plants in their home gardens. The information is limited because the crop is rather new for breeders and horticulturists. This study used fruit characters (fruit weight, length and width) and total soluble solid in immature, medium ripe and fully ripe stages to evaluate genetic variability in mulberry.

Based on CV values and F-ratios, high variations were observed in 21 varieties of mulberry collected in Thailand and Lao People's Democratic Republic for fruit weight, length and width and total soluble solid in immature, medium ripe and fully ripe stages. 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 find report on variations in fruit weight. However, fruit characters and total soluble solid has been used successfully in other related plant such as in Iranian pomegranate cultivars data were recorded for fruit weight (243.90 g), fruit length (75.86 mm) and total soluble solid(13.62 ºBrix) (Nemati *et al.*, 2012).

High F-ratio indicated that genotype contributed to large portion of total variation for fruit length, fruit width, and improvement of these traits will be easy. In this study, mulberry varieties with good fruit characters and high total soluble solid were identified, and these varieties 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 traits may affect other traits. In this study, fruit weight, length and width were inter-related with correlation coefficients ranging from 0.83 between fruit fresh weight and fruit width to 0.90 between fruit length and fruit width. Any fruit characters can be used as a selection criterion for fruit size depending on heritability of the characters and simplicity for measurement.

Total soluble solid values at immature, medium ripe and fully ripe stages were inter-related with correlation coefficients ranging from 0.22 between total soluble solid in immature stage and total soluble solid at medium ripe stage to 0.48 between total soluble solid at fully ripe stage and total soluble solid at medium ripe stage.

Total soluble solid at medium mature stage could be used for some extent as an indicator of total soluble solid at ripe stage. However, evaluation of total soluble solid at immature stage is not appropriate as it was not related to total soluble solid at ripe stage.

The correlations between fruit characters and total soluble solid at all stages were negative and significant. The results indicated that simultaneous improvement of fruit characters and total soluble solid is difficult although improvement of individual traits is easy as smaller fruit is associated with higher total soluble solid.

Cluster analysis

A dendrogram from cluster analysis based on fruit characters and total soluble solid divided 21 accessions of mulberry into 6 distinct clusters. A group of the accessions was not related with the origins of the accessions. This could be possibly due to the distribution of the accessions across geographic origins through migration of the people. People usually take plants or seeds with them when they relocate to other regions. However, molecular study of these accessions may confirm these observations.

In *Punica granatum* L., a valuable medicinal plant belongs to family Punicaceae, 31 cultivars were grouped into 5 clusters based on fruit morphological characteristics and total soluble solid (Nemati *et al.*, 2012).

The results indicated that horticultural traits and total soluble solid are useful parameters for evaluation of genetic diversity in mulberry. 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 mulberry 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 horticultural traits and total soluble solid to understand heterotic patterns of mulberry germplasm and to find appropriate ways for germplasm utilization.

CONCLUSION

This study reported variations for fruit fresh weight, length, width and total soluble solid in 21 accessions of fruit mulberry. High variations for this characters indicated that improvement of individual characters in this germplasm is possible. However, negative and significant correlations between fruit characters and total soluble solid indicated that improvement of mulberry for larger fruits and high total soluble solid may be difficult. The accessions were grouped into 6 different clusters using horticultural traits and total soluble solid. The groups with contrasting characters may be crossed to generate new segregating populations for selection of superior genotypes. This information will enable useful information for germplasm conservation, utilization and management for the development of mulberry in breeding programs.

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