



## Genetic differentiation and antioxidant activities of *Bouea macrophylla* Griffith in Nakhon Nayok province

Sirikul Thummajitsakul<sup>1</sup> · Kun Silprasit<sup>2</sup>

Received: 26 September 2016 / Accepted: 3 November 2016 / Published Online: 31 March 2017  
© The Korean Society for Applied Biological Chemistry 2017

**Abstract** Genetic differentiation and antioxidant activities in ethanolic extracts from leaves of *Bouea macrophylla* Griffith were determined. The result revealed genetic differentiation among sour ma-praang, ma-yong and sweet ma-praang of *B. macrophylla* Griffith ( $\phi_{PT}=0.772$ ,  $p$ -value=0.000). In addition, high genetic diversities were found in sour ma-praang and sweet ma-praang populations ( $P$ : 51.4 and 57.1 %;  $He$ : 0.1900.035 and 0.2240.036, respectively), but low genetic diversity was found in ma-yong population ( $P$ : 8.6 %;  $He$ : 0.0350.021). Total phenolic contents of sour ma-praang, ma-yong and sweet ma-praang were estimated as  $680.51\pm 89.81$ ,  $701.03\pm 59.89$  and  $530.85\pm 41.23$  mg gallic acid/g extract, respectively. Free radical scavenging activities of sour ma-praang, ma-yong and sweet ma-praang were found ( $1/EC_{50}=4.17$ , 1.43 and 1.37, respectively) corresponding with metal-chelating activities ( $1/EC_{50}=0.83$ , 0.65 and 0.17, respectively). Therefore, the obtained data may be applied to cultivation and utilization of their leaves as source of natural phenolics and antioxidants.

**Keywords** Antioxidant activity · *Bouea macrophylla* Griffith · Genetic differentiation · Random amplified polymorphic DNA

### Introduction

Marian plum (*Bouea macrophylla* Griffith) is a fruit tree that belongs to the family Anacardiaceae, and it is grown and widespread in Thailand and Sumatra (Blench 2008). In Thailand, this plant is a popular and important economic fruit tree, well known as ma-praang and ma-yong. Generally, it is classified according to its fruit tastes into 3 groups: sour ma-praang, sweet ma-praang, and ma-yong (Subhadrabandhu 2001). Furthermore, the plant characteristics have height at approximately 27 m and leaf shape like lanceolate or elliptic in length up to 45 cm and width up to 13 cm (Subhadrabandhu 2001). The unripe and ripe fruits of this plant have high nutritional values containing high potassium, essential amino acids, fiber and lipid (Rajan and Bhat 2016). In addition, it shows the existence of antioxidant compounds and antioxidant activities in unripe and ripe fruits (Rajan and Bhat 2016). Antioxidants can help to prevent several diseases that occur from oxidant compounds, such as cancer and alzheimer's disease (Halliwell et al. 2005; Valko et al. 2006; Gibson et al. 2008). Thus, antioxidants from many plants are good choices to use in food and health promoting of human. Due to most people worry about toxicities of artificial antioxidants used commonly in now.

Nevertheless, there are no scientific reports involving medicinal properties in leaves extracts and genetic structure of *B. macrophylla* Griffith. This work may help to promote cultivation and health for further commercial aspect. Therefore, random amplified polymorphic DNA (RAPD) were applied in this study. RAPD is one of popular molecular techniques using PCR method with a single arbitrary primer. This tool provides low cost, convenient, rapid and simplicity (Weder 2002; Suwanchaikasem et al. 2012). It has been used to distinguish and identify genotypes in several plant species (Arif et al. 2010; Thummajitasakul et al. 2014).

Nowadays, *B. macrophylla* Griffith is one of economical fruits but its leaves is not provided importance, in comparison with other plants. However, one of our major purposes is to find natural sources that may contain antioxidants which are useful in health promotion. In addition, some farmers and consumers lack

Sirikul Thummajitsakul (✉)  
E-mail: sirikul.thum@gmail.com

<sup>1</sup>Faculty of Physical Therapy, Srinakharinwirot University, Ongkharak, Nakhon-Nayok 26120, Thailand

<sup>2</sup>Faculty of Environmental Culture and Ecotourism, Srinakharinwirot University, Bangkok 10110, Thailand

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

knowledge in classification of *B. macrophylla* Griffith. Therefore, this study of genetic markers may assist in identification, breeding and conservation of *B. macrophylla* Griffith in Nakhon Nayok.

## Materials and Methods

### Sample collection

Leaves of *B. macrophylla* Griffith (Sour ma-praang, n=5; Sweet ma-praang, n=8; Ma-yong, n=56) were collected from 16 regions of Mueang Nakhon Nayok and Ban Na districts of Nakhon Nayok province in where the plant was popular to cultivate (Fig. 1). Furthermore, global positioning system was recorded by using UTM system. Width, length and tail of each leaf was measured, then cleaned by pure water and kept in a plastic bag at 20 °C until used.

### DNA extractions and RAPD method

Total DNA from fresh leaf of each sample was extracted using plant DNA extraction kit (RBC Bioscience, New Taipei City, Taiwan). Briefly, each sample tissue was grinded by a sterile pestle in 400 mL of GP1 buffer, then reacted with 5 mL of 10 mg/mL RNase A at 65 °C for 10 min. Next, GP2 buffer for 100 mL was mixed and left for 3 min at 4 °C. The supernatant was used further by adding GP3 buffer for 1.5 time. The purified DNA was obtained by applying to a GD column and centrifuged at 13,000 rpm for 3 min, then washed with 600 mL of W1 buffer and wash buffer, respectively, finally extracted with 100 mL of elution buffer. After that, RAPD reactions were carried out by a LifeECO thermal cycler (Bioer Technology, Hangzhou, China) according to Thummajitasakul et al. (2014a). Thirteen primers (Ashraf et al.

2014; Thummajitasakul et al. 2014a) were screened for RAPD analysis, of these, five primers (OPA03, OPA04, OPA09, OPA15 and C4) providing genetic polymorphisms were selected. Each RAPD condition was performed in a total volume of 20 L consisting of DNA template for 25 ng, 10 L of OnePCR Plus reaction mixture which contained fluorescence dye, Taq DNA polymerase, PCR buffer, dNTP and loading dye (GeneDireX, Las Vegas City, NV, USA), and 1 μM of each arbitrary primer. The reaction was run as one cycle of initial denaturation at 95 °C for 5 min, followed by 35 cycles of DNA denaturation at 95 °C for 30 s, annealing at 45 °C for 1 min and extension at 72 °C for 1 min. Finally, the last extension was at 72 °C for 5 min, then cooled down at 4 °C. The PCR products were checked on a 1.5 % electrophoresis gel in 0.5XTAE buffer (Pacific Science Co., Ltd., Bangkok, Thailand) at 100 volts for 20 min, and visualized under UV light (Omega Fluor, Aplegen). A 100 bp DNA ladder containing fluorescence and loading dye was used as marker (GeneDireX).

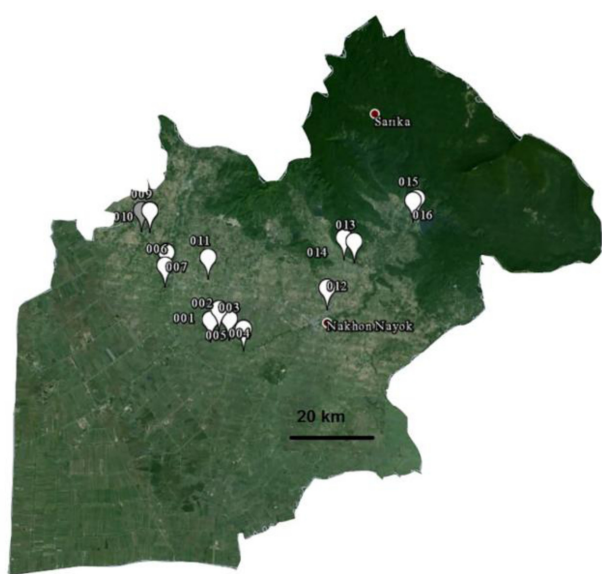
### Sample extracts and total phenolic contents

The fresh leaves from sour ma-praang, sweet ma-praang, and ma-yong of *B. macrophylla* Griffith were washed by distilled water and incubated at 50 °C until dry. After that, each sample was grinded to a powder by a homogenizer, and kept in a plastic bag at room temperature until analyzed. To obtain extracts, 15 g of dried powder was extracted with 150 mL of 95 % ethanol solvent at 50 °C for 16 h in an incubator with shaking. Then, the solvent was evaporated in the incubator at 50 °C until dry and adjusted the initial concentration into 100 mg/mL. Each extract was collected and kept at 20 °C until used. Each extraction was carried out in duplicate.

Total phenolic contents were estimated by the Folin-Ciocalteu colorimetric method (Deetae et al. 2012). Each extract (300 L) was reacted with 1.5 mL of Folin-Ciocalteu reagent for 5 min, and added 1.2 mL of sodium carbonate (7.5 % w/v) with incubation at room temperature for 30 min. Each reaction was performed in duplicate. Next, an absorbance was determined at wavelength 765 nm by spectrophotometer (Model T60UV). Water and gallic acid (0-100 g/mL) were used as blank and a standard phenolic compound, respectively. Total phenolic contents were compared in unit of mg gallic acid equivalent per gram extract.

### Antioxidant activities

The antioxidant activity was performed via an 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical method (Deetae et al. 2012). First, the ABTS free radical cation was produced from a reaction of 7 mM ABTS solution (10 mL) with 140 mM potassium persulfate (179 μL) in dark condition at room temperature for 12–16 h. To assay antioxidant activity, the ABTS radical solution was diluted with aqueous solvent to obtain an absorbance of 0.700±0.050 at 734 nm. Next, 3.9 mL of the diluted solution was added in 20 μL of each plant extract and mixed



**Fig. 1** Sampling map for *B. macrophylla* Griffith in Nakhon Nayok province

gently, then allowed to stand for 6 min at room temperature in dark condition. After that, the absorbance was determined immediately at 734 nm. Each reaction was tested in duplicate. The percentage of antioxidant capacity was calculated following the method of Thummajitasakul et al. (2014b).

Moreover, a ferrous ion-chelating (FIC) assay of each sample was detected with the protocol of Deetae et al. (2012). Each extract (1 mL) was added with 0.1 mM FeSO<sub>4</sub> (1 mL) and 0.25 mM ferrozine (1 mL), and incubated for 10 min in dark condition at room temperature. Then, an absorbance was determined at 562 nm with EDTA used as the positive control. Each reaction was tested in duplicate. The percentage of metal chelating ability was estimated according to the method of Thummajitasakul et al. (2014b). The effective concentration (EC) 50 value, which was an effective concentration of antioxidants at 50 % of the free radical scavenging or metal chelating capacities, was calculated from the graph. Then, the EC50 value was modified to 1/EC50 because this value varies directly according to its antioxidant activity

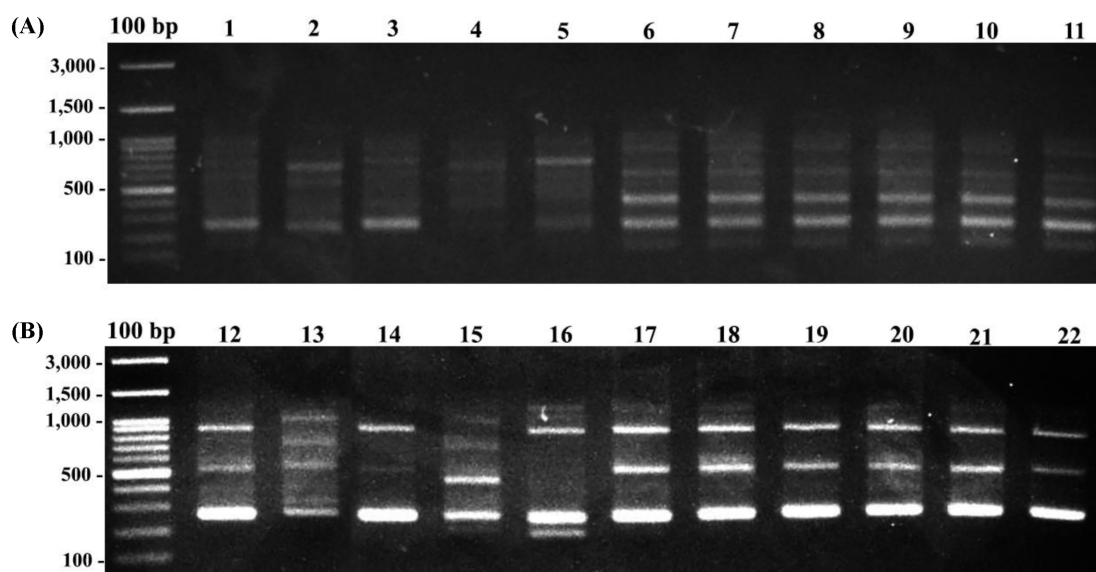
#### Data analysis

A binary data was scored with the presence (1) or absence (0) of a RAPD band. After that, the values of genetic diversity were determined by haplotype patterns, mean expected heterozygosity (*He*) and percentages of polymorphic bands (*P*). The molecular variances among populations (sour ma-praang, sweet ma-praang, and ma-yong) and between two groups (group 1: sour ma-praang and sweet ma-praang, and group 2: ma-yong) of *B. macrophylla* Griffith were analyzed by AMOVA using GenAlEx 6.51 program (Peakall and Smouse 2006; Peakall and Smouse 2012). The principal component analysis (PCA) was performed using excel add-in Multibase version 2015 (Numerical Dynamics 2009).

Analysis of variance (ANOVA) method was used to estimate the difference of antioxidant activities and leaf size (width, length and tail) among three populations at significant level of *p*-value <0.05 (Soper 2016).

#### Results and Discussion

The RAPD is one of effective techniques having usefulness for many studies in genetic diversities and characterization of several fruit trees, i.e. *Dimocarpus longan* Lour. (Mei et al. 2014), *Litchi chinensis* Sonn. (Cheng et al. 2015). The result showed the RAPD patterns from each selected primer and total 35 loci, and 18 haplotype patterns were obtained (Fig. 2). To elucidate whether genetic differentiation was found in this plant, total samples were divided into 3 populations (sour ma-praang, sweet ma-praang, and ma-yong). High genetic diversities were detected within sour ma-praang and sweet ma-praang providing *p* values for 51.4 and 57.1 %, and *He* values for 0.1900.035 and 0.2240.036, respectively. Moreover, low genetic diversity was found in ma-yong population (*p* values =8.6 % and *He* =0.0350.021) shown in Table 1. The result indicated relatively low level in genetic variation of ma-yong population in comparison to other populations. It may result from cumulative inbreeding within population and habitat restriction (Ellstrand and Elam 1993; Glover and Abbott 1995). In addition to the result above, mean difference of width, length and leaf tail among populations was analyzed by one-way ANOVA. Although, it showed that sour ma-praang and sweet ma-praang were insignificantly detected in size difference for leaf width, length and tail length (*p*-value =0.874, 0.504, and 0.524, respectively), their leaf sizes were larger than ma-yong, significantly (*p*-value



**Fig. 2** Examples of RAPD patterns amplified by primer OPA04 (A) and primer OPA03 (B). Lanes 1-3, 12-14 were sweet ma-praangs, lanes 4-5 and 15-16 were sour ma-praangs, and lanes 6-11 and 17-22 were ma-yongs of *B. macrophylla* Griffith. The 100 bp DNA ladder was used as marker

**Table 1** Genetic diversity and mean leaf sizes within each population (sour ma-praang, sweet ma-praang and ma-yong)

Samples	He	P (%)	No. of haplotypes	No. of Different Bands	No. Private Bands*	Leaf sizes [Mean ± SD (cm)]		
						Leaf width	Leaf length	Length of leaf tail
Sour ma-praang (n=5)	0.190±0.035	51.4	5	27	0	4.50±0.50	16.74±2.16	1.34±0.50
Sweet ma-praang (n=8)	0.224±0.036	57.1	8	27	1	4.44±0.72	15.6±3.24	1.56±0.63
Ma-yong (n=56)	0.035±0.021	8.6	5	22	3	3.02±0.88	10.15±2.50	0.79±0.35
Total (n=69)	0.204±0.035	68.6	18	31	31			

\*No. Private Bands=No. of Bands Unique to a Single Population

**Table 2** Genetic differentiation among sour ma-praang, sweet ma-praang and ma-yong populations, and between 2 groups (group 1: sour ma-praang and sweet ma-praang, and group 2: ma-yong), which were analyzed by AMOVA

	Source	df	SS	MS	Est. Var.		
3 populations	Among Pops	2	88.417	44.208	3.869	$f_{PT}=0.772$	$p\text{-value}=0.000^*$
	Within Pops	66	75.496	1.144	1.144		
3 populations, 2 groups	Among Regions	1	84.197	84.197	3.554	$f_{RT}=0.684$	$p\text{-value}=0.000^*$
	Among Pops	1	4.219	4.219	0.500	$f_{PR}=0.304$	$p\text{-value}=0.017^*$
	Within Pops	66	75.496	1.144	1.144	$f_{PT}=0.780$	$p\text{-value}=0.000^*$

\*The mean difference is significant at  $p\text{-value} < 0.05$

=0.000) (Table 1). For AMOVA analysis, high genetic differentiation among three populations was significantly found ( $\phi_{PT}=0.772$ ,  $p\text{-value}=0.000$ ). While the populations were divided into 2 groups (group 1: sour ma-praang and sweet ma-praang, and group 2: ma-yong), high genetic variance between two groups was also found ( $\phi_{RT}=0.684$ ,  $\phi_{PT}=0.304$ ,  $\phi_{PR}=0.780$ ;  $p\text{-value}=0.000$ , 0.017 and 0.000, respectively) (Table 2). Therefore, the AMOVA result provided high genetic differentiation among populations; sour ma-praang, sweet ma-praang and ma-yong. Of these, ma-yong showed genetic differentiation which was separated from sour ma-praang and sweet ma-praang groups. Moreover, one-way ANOVA result confirmed that leaf width, length and tail length of sour ma-praang and sweet ma-praang groups were bigger than that of ma-yong. Because all ma-yong samples, collected in this study well known “ma-yong chid, are popular and become to mainly cultivate in Nakorn Nayork area, it may affect to lost genetic diversity in this population. To date, there are not exactly in classification of *B. macrophylla* Griffith. Most people recognized *B. macrophylla* Griffith according to fruit tastes into 3 groups, i.e. sour ma-praang, sweet ma-praang, and ma-yong (Subhadrabandhu 2001). In addition, based on reproduction by farmers in native areas, an origin of ma-yong chid was propagated from young sour ma-praang produced by seeding (Subhadrabandhu 2001). Then, new high quality clones providing sweet taste with some sourness were selected for further commercialization and cultivation. Thus, it suggested that they may be mutated until they provide good taste and become widespread for consumers.

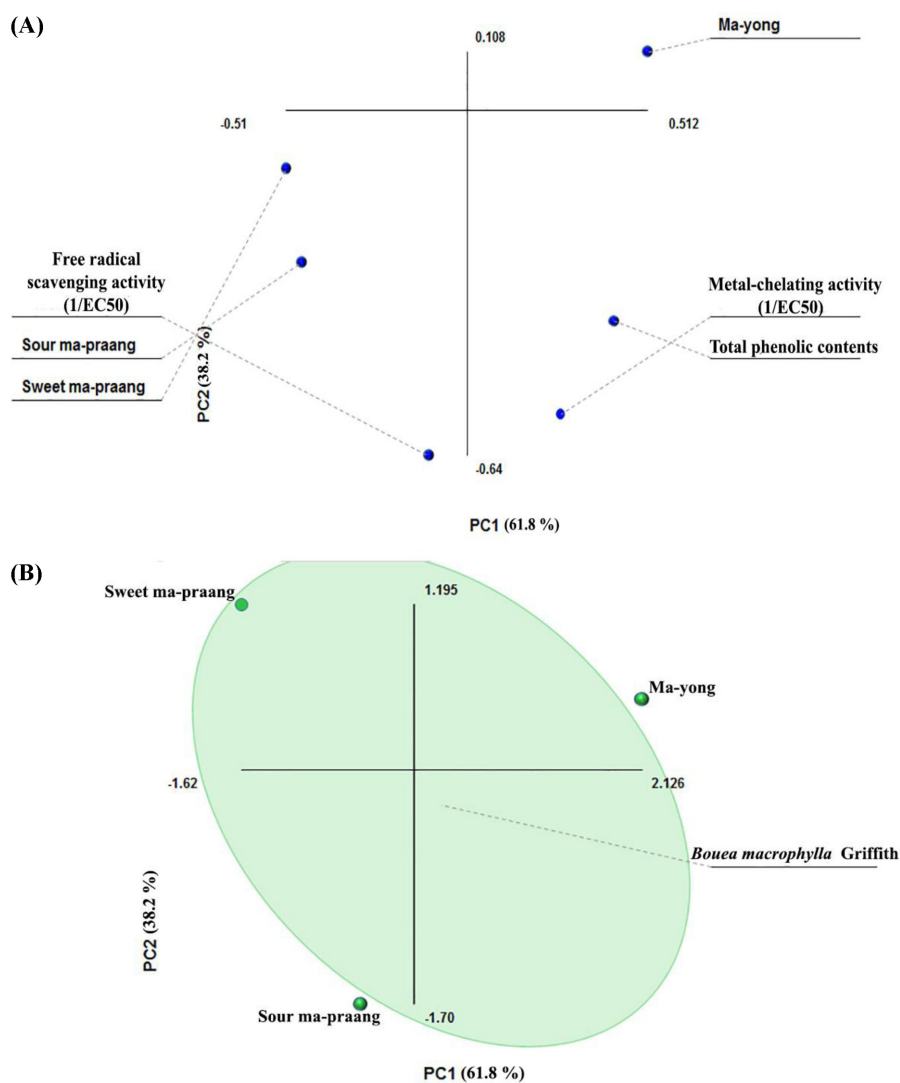
Moreover, total phenolic contents in 95 % ethanol extracts showed insignificantly difference between sour ma-praang and ma-yong ( $p\text{-value}=0.717$ ), but their total phenolic contents revealed higher quantities than that of sweet ma-praang, significantly ( $p\text{-value}=0.023$  and 0.003, respectively). Several researches found

many phenolics in fruits and plants more than 8,000 compounds with known structures, such as phenols, phenolic acids, flavonoids and tannins (Harbone and Williams 2000; Santos-Buelga and Sacalbert 2000; Naczki and Shahidi 2006). In this work, total phenolic contents were found in 95 % ethanol extracts from leaves of sour ma-praang, sweet ma-praang and ma-yong. The result indicated total phenolic contents in sour ma-praang and ma-yong higher than sweet ma-praang. There were many reports in other plants with sour taste, such as Mango (*Mangifera indica* L.) had some phenolic compounds, e.g. high concentration of mangiferin in young leaves (Barreto et al. 2008). Besides these, leaves extracts of citrus plants revealed high level of phenolic compounds (Al-Anbari and Hasan 2015).

Moreover, EC50 values of free radical scavenging activity in each extract between sweet ma-praang and ma-yong showed insignificantly difference ( $p\text{-value}=0.539$ ), and showed higher value than that of sour ma-praang, significantly ( $p\text{-value}=0.000$ ). For metal-chelating activity, it found that EC50 values between sour ma-praang and ma-yong showed insignificantly difference ( $p\text{-value}=0.164$ ), and showed lower values than that of sweet ma-praang, significantly ( $p\text{-value}=0.003$  and 0.005, respectively) (Table 3). Many researches have reported antioxidant activities from plant extracts involving phenolic compounds. Of these, polyphenolics can play a major role as antioxidant activity (Lima et al. 2014), such as flavonoids, led to useful for human health by free radical scavenging and metal chelating properties (Harbone and Williams 2000; Mira et al. 2002). In this study, free radical scavenging activity was determined by ABTS method, and metal-chelating activity was estimated by FIC method. The principle of the ABTS method is the production of the ABTS free radical cation for using in reduction reaction with antioxidants, and it can be applied to determine high-pigmented, hydrophilic and lipophilic

**Table 3** Total phenolic contents, free radical scavenging activities and metal-chelating activities in 95 % ethanol extracts of sour ma-praang, sweet ma-praang and ma-yong leaves

Samples	Total phenolic contents (mg gallic acid/ g extract)	ABTS		FIC	
		EC <sub>50</sub>	1/EC <sub>50</sub>	EC <sub>50</sub>	1/EC <sub>50</sub>
Sour ma-praang	680.51±89.81	0.24±0.08	4.17	1.21±0.1	0.83
Sweet ma-praang	530.85±41.23	0.73±0.07	1.37	5.75±0.35	0.17
Ma-yong	701.03±59.89	0.7±0.06	1.43	1.55±0.2	0.65



**Fig. 3** The PCA plots. A was the plot of variables (loading) for total phenolic contents, free radical scavenging activity, metal-chelating activity, and the genetic similarity values among sour ma-praang, sweet ma-praang and ma-yong. B was the plot of samples (score) for sour ma-praang, sweet ma-praang and ma-yong of *Bouea macrophylla* Griffith

antioxidants (Floegel et al. 2011). For FIC method, metal-chelating activity is based on the prevention of the association between metal and ferrozine (Robu et al. 2012). The chelating activity has a role in treatment of metal overload condition in human body, such as Thalassemia (Ebrahimzadeh et al. 2008). In this study, the ethanol leaves extracts of *B. macrophylla* Griffith, showed free radical scavenging and metal-chelating activities.

Sour ma-praang showed the lowest EC50 value indicating the highest of free radical scavenging activity. Moreover, metal-chelating activities were mostly found in leaves extracts of sour ma-praang and ma-yong. However, plant extracts may content some non-phenolic antioxidants that play importance to contribute antioxidant activities, such as the report of el-Sayed et al. (2008) found four non-phenolic antioxidants (i.e. 1-O-beta-D-glucopyranosyl-

2-methoxy-3-(2-hydroxy-triaconta-3,12-dienoate)-glycerol) in leaves extracts of *Buddleja asiatica* Lour. In addition, several antioxidant agents (phenolics, flavonoids, flavonols, tannins, anthocyanins and ascorbic acid) of unripe and ripe fruit extracts of *B. macrophylla* Griffith have been detected in different solvent extracts (methanol, ethanol, and distilled water) according to the literature (Rajan and Bhat 2016). Of these solvents, the highest antioxidant activity was found in methanol extracts of unripe fruits (Rajan and Bhat 2016). However, there were several factors to increase phenolic contents in plants, such as stressful environments (e.g. insect attack and wound) and organic planting (Woese et al. 1997; Reyes and Cisneros-Zevallos 2003; Mitchell et al. 2007).

The results were confirmed with distribution pattern among three populations of *B. macrophylla* Griffith of PCA shown in the loading plot using the values of genetic similarities obtained from RAPD analysis. Moreover, interrelation among total phenolic contents, free radical scavenging activity, metal-chelating activity were demonstrated with 1/EC50 values in the loading PCA plot. The PC1 and PC2 revealed 61.8 and 38.2 % of the total variance, respectively. PC1 loadings had total phenolic contents, and three populations, while PC2 loading consisted of free radical scavenging and metal-chelating activities. When the loading and score plots were overlaid, it revealed that sour ma-praang and sweet ma-praang had clustered together, but ma-yong was separated in the plot. Moreover, the ethanolic extract of sour ma-praang showed the highest of total phenolic contents, free radical scavenging and metal-chelating activities (Fig. 3). The PCA result clearly indicated that genetic differentiation existing among sour ma-praang, sweet ma-praang and ma-yong, and it suggested that sour ma-praang showed genetic similarity with sweet ma-praang more than ma-yong. Furthermore, it also confirmed that total phenolic contents, free radical scavenging and metal-chelating activities were mostly found in leaves extracts of sour ma-praang.

In conclusion, based on our research, genetic differentiation was detected among sour ma-praang, sweet ma-praang, and ma-yong of *B. macrophylla* Griffith. Moreover, high genetic diversities were found within population of sour ma-praang and sweet ma-praang, but low genetic diversity was found in ma-yong population. RAPD technique was certificated to be useful for genetic analysis. It also concluded that high amounts of total phenolic contents, free radical scavenging and metal-chelating activities were detected in leaves extracted with 95 % ethanol solvent. Therefore, more studies are need to identify sequence related amplified polymorphism, bioactive compounds and to extend scope in its cultivation to rise phenolic contents before economical applications, and pharmaceutical useful as natural sources of antioxidants.

**Acknowledgment** The authors thank a grant from Srinakharinwirot University (Grant number 780/2558), Faculty of Physical Therapy of Srinakharinwirot University for supporting this work, and also acknowledge Faculty of Environmental Culture and Ecotourism, Srinakharinwirot University for research facilities. The authors have no conflicts of interest in this research.

## References

- Al-Anbari AKH, Hasan MA (2015) Antioxidant Activity in Some Citrus Leaves and Seeds Ethanolic Extracts. International Conference on Advances in Agricultural, Biological & Environmental Sciences (AABES-2015), London
- Arif IA, Bakir MA, Khan HA, Al Farhan AH, Al Homaidan AA, Bahkali AH, Al Sadoon M, Shobrak M (2010) Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. *Genet Mol Res* 9: 2191–9198
- Ashraf K, Ahmad A, Chaudhary A, Mujeeb M, Ahmad S, Amir M, Mallick N (2014) Genetic diversity analysis of *Zingiber Ocinale* Roscoe by RAPD collected from subcontinent of India. *Saudi J Biol Sci* 21: 159–165
- Barreto JC, Trevisan MTS, Hull WE, Erben G, De Brito ES, Pfundstein B, Wurtele G, Spiegelhalder B, Owen RW (2008) Characterization and Quantitation of Polyphenolic Compounds in Bark, Kernel, Leaves, and Peel of Mango (*Mangifera indica* L.). *J Agric Food Chem* 56: 5599–5610
- Blench R (2008) A history of fruits on the Southeast Asian mainland. Kay Williamson Educational Foundation, Cambridge
- Cheng J, Long Y, Khan MA, Wei C, Fu S, Fu J (2015) Development and significance of RAPD-SCAR markers for the identification of *Litchi chinensis* Sonn. by improved RAPD amplification and molecular cloning. *Electron J Biotechnol* 18: 35–39
- Deetae P, Parichanon P, Trakunleewatthana P, Chanseetis C, Lertsiri S (2012) Antioxidant and anti-glycation properties of Thai herbal teas in comparison with conventional teas. *Food Chem* 133: 953–959
- Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR (2008) Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. *Afr J Biotechnol* 7: 3188–3192
- el-Sayed MM, el-SS AH, Ahmed WS, el-Wakil EA (2008) Non-phenolic antioxidant compounds from *Buddleja asiatica*. *Z Naturforsch C* 63: 483–491
- Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. *Annu Rev Ecol Syst* 24: 217–242
- Floegel A, Dae-Ok K, Sang-Jin C, Koo SI, Chun OK (2011) Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J Food Compost Anal* 24: 1043–1048
- Gibson GE, Karuppagounder SS, Shi Q (2008) Oxidant induced changes in mitochondria and calcium dynamics in the pathophysiology of Alzheimer's disease. *Ann N Y Acad Sci* 1147: 221–232
- Glover BJ, Abbott RJ (1995) Low genetic diversity in the Scottish endemic *Primula scotica* Hook. *New Phytol* 129: 147–153
- Halliwel B, Rafter J, Jenner A (2005) Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not?. *Am J Clin Nutr* 81: 268–276
- Harbone JB, Williams C (2000) Advances in flavonoid research since 1992. *Phytochemistry* 55: 481–504
- Lima GPP, Vianello F, Corrêa CR, da Silva Campos RA, Borguini MG (2014) Polyphenols in Fruits and Vegetables and Its Effect on Human Health. *Food Nutr Sci* 5: 1065–1082
- Mei ZQ, Fu SY, Yu HQ, Yang LQ, Duan CG, Liu XY, Gong S, Fu JJ (2014) Genetic characterization and authentication of *Dimocarpus longan* Lour. using an improved RAPD technique. *Genet Mol Res* 13: 1447–1455
- Mira L, Fernandez MT, Santos M, Rocha R, Florêncio MH, Jennings KR (2002) Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. *Free Radic Res* 36: 1199–1208
- Mitchell AE, Hong YJ, Koh E, Barret DM, Bryant DE, Denison RF, Kafka S (2007) Ten-Year Comparison of the Influence of Organic and Conventional Crop Management Practices on the Content of Flavonoids in Tomatoes. *J Agric Food Chem* 55: 6154–6159
- Nacz M, Shahidi F (2006) Phenolics in cereals, fruits and vegetables:

- occurrence, extraction and analysis. *J Pharm Biomed Anal* 41: 1523–1542
- Numerical Dynamics (2009) The Excel add-in Multibase package. <http://www.numericaldynamics.com>. Accessed 12 February 2016
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6: 288–295
- Peakall R, Smouse PE (2012) GenAIEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537–2539
- Rajan SN, Bhat R (2016) Antioxidant compounds and antioxidant activities in unripe and ripe kundang fruits (*Bouea macrophylla* Griffith). *Fruits* 71: 41–47
- Reyes LF, Cisneros-Zevallos L (2003) Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoess (*Solanum tuberosum* L.). *J. Agric Food Chem* 51: 5296–5300
- Robu S, Aprotosoie AC, Miron A, Cioanca O, Stanesuc U, Hancianu M (2012) Antioxidant activity of ethanolic extracts from some lavandula species cultivated in Romania. *Farmacia* 60: 394–401
- Santos-Buelga C, Sacalbert A (2000) Proanthocyanidins and Tannin-Like Compounds Nature, Occurrence, Dietary Intake and Effects on Nutrition and Health. *J Sci Food Agr* 80: 1094–1117
- Soper D (2016) Analysis of Variance (ANOVA) Calculator-one-way ANOVA from summary data. <http://www.danielsoper.com/statcalc/default.aspx>. Accessed 12 February 2016
- Subhadrabandhu S (2001) Under-utilized tropical fruits of Thailand. FAO Regional Office for Asia and the Pacific, Thailand
- Suwanchaikasem P, Chaichantipyuth C, Amnuoyopol S, Sukrong S (2012) Random amplified polymorphic DNA analysis of *Thunbergia laurifolia* Lindl. and its related species. *J Med Plant Res* 6: 2955–2961
- Thummajitasakul S, Deetae P, Silprasit K (2014a) Genetic polymorphisms of *Stachytarpheta jamaicensis* (L.) Vahl based on a high annealing temperature RAPD (HAT-RAPD). *SWU Sci J* 30: 77–87
- Thummajitasakul S, Tumchalee L, Koolwong S, Deetae P, Kaewsri W, Lertsiri, S (2014b) Antioxidant and antibacterial potentials of some Thai native plant extracts. *Int Food Res J* 21: 2393–2398
- Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 160: 1–40
- Weder JK (2002) Identification of plant food raw material by RAPD-PCR: legumes. *J Agric Food Chem* 50: 4456–4463
- Woese K, Lange D, Boess C, Bögl KW (1997) A Comparison of Organically and Conventionally Grown Foods-Results of a Review of the Relevant Literature. *J Sci Food Agr* 74: 281–293