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Variation of Total Curcuminoids Content, Antioxidant Activity and Genetic Diversity in Turmeric (*Curcuma longa* L.) Collections

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ABSTRACT

Turmeric has recently been developed as an antiulcer drug and as a mosquito repellant on a commercial scale. However, the material supplied to the factory has not been uniform and has contained lower amounts of active compound than specified in the Thai Herbal Pharmacopoeia. The objective of this study was to reveal the genetic diversity and variation in active compounds and the bioactivity of turmeric collected from different parts of Thailand. The total curcuminoids content and antioxidant activity of 67 samples of *Curcuma longa* L. and one sample of *C. mangga* rhizome from various locations were analyzed before and after planting. The highest total curcuminoids content was found in the samples from the central region while the lowest content was found in the samples from the Lao PDR The antioxidant activities of extracts were assayed with DPPH and compared with ascorbic acid. The highest antioxidant activity (EC_{50} 8.04±3.77 mg/ml) was found in the samples from the central region, which was highly correlated with the curcuminoids content. The antioxidant activity of turmeric from all regions decreased after planting for six months. The RAPD technique was performed to detect genetic diversity in the turmeric samples. Nineteen RAPD primers yielded 184 bands of which 166 were polymorphic. At 68.4% genetic similarity, the samples were separated into four groups. The results indicated the possibility of selecting high quality clones for large-scale production.

Key words: *Curcuma longa* L., turmeric, curcuminoids, antioxidant activity, RAPD

INTRODUCTION

The rhizomes of turmeric (*Curcuma longa* L., Zingiberaceae) are commonly used as a flavoring, coloring agent and preservative. Commercially, it is traded as a dye, spice and source of industrial starch. The characteristic yellow-orange curcuminoids found in rhizomes are used for coloring food and textiles. Recently, it has attracted much attention due to its significant medicinal potential (Cousins *et al*., 2007).

The main yellow bioactive substances in the rhizomes are due to curcumin, demethoxycurcumin and bisdemethoxycurcumin. Many studies have reported the biological, physiological and chemical properties of turmeric. Turmeric has been reported to possess anti-inflammatory, hepatoprotective, antitumor, antiviral activities (Ammon and Wahl, 1991) and anticancer activity (Polasa *et al*., 1991) and is used in gastrointestinal and respiratory disorders (Anwarul *et al*., 2006). Curcuminoids exhibit free-radical scavenging properties, antioxidant activity (Toda *et al*., 1985; Soudamini, 1989; Ammon *et al*., 1993; Selvam *et al*., 1995; Masuda *et al*., 1999) and act as inhibitors of human immune deficiency virus type

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1 (HIV-1) integrase (Mazumder *et al.*, 1995). Turmeric oil is composed of several monoterpene and sesquiterpene compounds such as zingiberene; ar-turmerone, α-turmerone and β-turmerone (Apisariyakul *et al.*, 1995). The main biological activities of the oil are carminative, antiflatulence, antifungal and as an antiplatelet agent (Lee, 2006). The Thai Herbal Pharmacopoeia (THP) recommended that dried turmeric should contain not less than 6.0% v/w of turmeric oil and 5.0% w/w of total curcuminoids (THP, 1995).

Free radicals were a major interest for early physicists and radiologists and much later, the free radicals were found to be a product of normal metabolism. Today, it is well known that radicals cause molecular transformations and gene mutations in many types of organisms. Recent data suggest that curcumin and other antioxidant products from the dried rhizome of turmeric may be useful in the prevention or treatment of some age-related degenerative processes (Miquel *et al.*, 2002).

The use of turmeric is spreading internationally and its production must be increased to meet the national and international demand by an increase in both the area of cultivation and the yield per unit area. In order to introduce turmeric cultivation into non-traditional areas, cultivars that are adapted to specific agroclimates and give high yields need to be identified.

Knowledge of genetic variability is essential for breeding programs and plant genetic resource conservation. Molecular marker techniques overcome many of the limitations of morphological and biochemical techniques since they are not affected by the environment or developmental stage and can detect variation at the DNA level (Tingey and Tufo, 1993).

This study was undertaken to verify the variation of total curcuminoids and antioxidant activity in turmeric rhizomes collected from different parts of Thailand before and after planting and to study genetic diversity using the RAPD technique.

MATERIALS AND METHODS

Plant materials

Rhizomes of *C. longa* L. were collected from 66 different locations in Thailand: North (10 accessions), Northeast (16 accessions), Central (8 accessions), West (3 accessions), South (29 accessions), and Lao P.D.R. (1 accession) plus one accession of *Curcuma mangga*. The samples were divided in two batches. The first batch was used for a pre-planting experiment and the second batch was planted in May in an experimental field of the Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. They were harvested at six months after planting for the second experiment.

Assay for total curcuminoids content

Fresh rhizomes were cleaned and sliced into small pieces and air dried for two days. The samples were further dried in a hot-air oven at 50°C for 24 h and then ground into powder. Total curcuminoids content was calculated using a standard curve (Figure 1). Analysis of each sample was carried out in triplicate.

Standard solution was prepared according to the method of Boonchoong *et al.* (2006). Standard curcumin (2.00 mg) was accurately weighed and transferred to a 5-ml volumetric flask. Distilled methanol was added and adjusted to a final concentration of 400 μg/ml. From this solution, concentrations of 0.4, 0.8, 1.6, 2.0, 2.4, 3.2 and 4.0 μg/ml were prepared and used for preparation of the calibration curve. For preparation of the sample solution from turmeric powder, the powder (100.00 mg) of each sample was separately transferred to a 10-ml volumetric flask, adjusted to 30 μg/ml and measured at 420 nm.

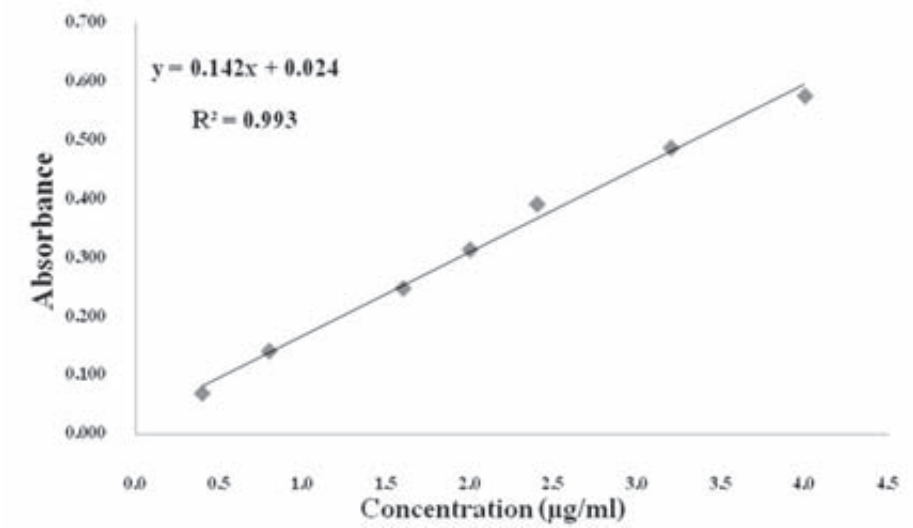

Figure 1 Calibration curve of the curcumin standard in methanol determined by spectrophotometer.

Assay for DPPH radical scavenging activity
Plant extraction

The modified extraction method of Chirangini *et al*. (2004) was used with one gram of fresh rhizome ground using pestle and mortar with liquid nitrogen, and then 10 ml of 95% distillable ethanol was added before centrifuging at 6000 rpm for 10 min. Finally, the clear sample was filtered using Whatman No. 4 filter paper and kept in a capped bottle at -20°C until used for antioxidant activity assay.

DPPH radical scavenging activity

The evaluation of radical-scavenging activity (antioxidant activity) was conducted by the method of Blois (1958) with modifications. A stock solution of the sample (100 mg/ml) was diluted for five concentrations. Each concentration was tested in triplicate. The portion of sample solution (0.5 ml) was mixed with 3.0 ml of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH, in 95% distillable ethanol) and allowed to stand at room temperature for 20 min under light protection. The absorbance (A) was measured at 517 nm. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The results were expressed as a percentage of inhibition. Ethanol was used as the control and the results were compared with the activity of a known antioxidant, ascorbic acid (Equation 1):

$$\% \text{ inhibition} = (1-(A_{sample}/A_{blank})) \times 100 \quad (1)$$

where A_{sample} = absorbance of sample with DPPH
A_{blank} = absorbance of control with DPPH

In the DPPH test, antioxidants were typically characterized by their EC_{50} value (effective concentration of sample required to scavenge 50% of DPPH radicals). The results were obtained by linear regression analysis of the dose-response curve plotted using % inhibition and concentration.

Genetic diversity using RAPD technique
DNA extraction

Young fresh leaves of turmeric were used for the isolation of DNA. The genomic DNA was isolated by the CTAB method (Doyle and Doyle, 1990).

RAPD primers

A total of 19 random primers (OPA-02, OPA-03, OPA-04, OPA-08, OPA-09, OPA-15, OPA-18, OPJ-09, OPR-01, OPR-02, OPR-07, OPR-08, OPR-13, OPR-15, OPS-11, OPS-12, OPT-05, OPT-08, and OPT-20) were selected for RAPD analysis from Operon Technologies, Inc., Almeda, USA.

PCR condition

RAPD reaction was carried out with a 20 μl reaction volume containing 25 ng genomic DNA, 1 U *Taq* DNA polymerase (Vivantis), 200 mM dNTPs, 2 mM $MgCl_2$ and 10 pmoles of random primer using the 19 primers. Amplification conditions consisted of pre-denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 37°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min.

Electrophoresis of PCR products

The amplified products were separated in a 0.8% agarose gel containing 0.5 mg ml^{-1} of ethidium bromide and visualized by a gel documentation system. The bands were scored based on 1 kb DNA ladder marker.

Data scoring and analysis

The DNA bands were scored with regard to polymorphic band individual lanes being either present (1) or absent (0). The NTSYS pc program version 2.20k was used to estimate genetic similarities with the simple matching coefficient. The matrix of generated similarities was analyzed by the unweighted-pair group method with arithmetic average (UPGMA), using the SAHN clustering module.

Principal component analysis (PCA) was also performed using the NTSYS program. Three-dimensional ordination provided an additional representation of genetic relationships among the individuals in the population. This multivariate approach was chosen to complement the cluster analysis information because cluster analysis is more sensitive to closely related individuals, whereas PCA is more informative regarding distances between major groups.

Statistical analysis

Total curcuminoids content and antioxidant activity were expressed as the mean±S.D. (n=3). Significance of difference was calculated by Duncan's new multiple range test and results with $P<0.05$ were considered statistically significant. To analyze the correlation between total curcuminoid content and antioxidant activity, Pearson's correlation coefficient was calculated.

RESULTS AND DISCUSSION

Total curcuminoids content

Total curcuminoids content was analyzed in distilled methanol and measured at 420 nm. Total curcuminoids content of the samples analyzed before planting ranged from 0.32±0.44 to 10.13±1.27% w/w. Only 40 of the 67 turmeric samples were within the limits recommended by THP. Sample S29 contained the highest content of total curcuminoids, while NE8 had the lowest. Total curcuminoids content in six-month-old rhizomes varied from 0.46±0.08 to 12.39±0.3% w/w, with only 35 of the 67 turmeric samples being within the limits recommended by THP. Sample S20 contained the highest content, while NE8 was the lowest (Table 1).

The total curcuminoids content of the samples before and after planting from each region were significantly different. The samples from the North, Northeast and Lao contained less than 5% w/w dried powder, which is below the amount recommended by THP. The samples from Central contained the highest content. The lowest content was obtained from the Lao sample as well as from *C. mangga*. A similar result was found in the six-

Table 1 Total curcuminoids content and EC_{50} value of all turmeric accessions before and after planting for six months (expressed as the mean±SD from sampling in triplicate).

		Total curcuminoids (% w/w)		EC_{50} (mg/ml)	
Location	Code	Before planting	After planting	Before planting	After planting
North	N1	1.68±0.42	1.95±0.34	14.55±5.15	26.11±7.90
	N2	2.60±0.33	2.46±1.02	13.10±4.10	13.86±4.82
	N3	6.15±1.59	4.83±1.24	7.29±2.04	13.42±1.17
	N4	6.37±0.47	4.42±1.02	9.84±2.36	18.40±2.14
	N5	6.81±1.35	2.97±0.94	10.20±3.44	13.28±1.06
	N6	4.87±0.93	3.33±1.02	9.83±1.46	12.83±0.78
	N7	4.95±0.84	3.68±1.86	13.61±1.49	17.53±5.51
	N8	7.38±1.27	6.05±2.38	9.23±2.93	11.38±6.51
	N9	4.03±0.55	6.88±2.67	19.03±13.48	16.30±6.73
	N10	4.14±0.62	5.26±1.24	6.80±3.09	19.90±4.14
	Average	4.94±1.94	4.18±1.99	11.39±5.61	16.30±5.82
Northeast	NE1	3.80±0.26	2.44±1.09	8.03±2.99	23.81±5.05
	NE2	4.23±0.66	6.13±0.47	7.29±2.04	12.65±4.06
	NE3	5.99±0.48	5.02±2.62	19.28±10.84	8.53±1.37
	NE4	4.96±0.49	6.25±0.81	6.14±1.02	14.15±1.90
	NE5	0.32±0.44	0.73±0.21	43.82±1.97	75.67±40.79
	NE6	6.74±0.62	7.35±0.34	6.24±0.98	11.80±1.66
	NE7	0.92±0.59	0.76±0.25	63.78±23.15	77.23±37.29
Northeast	NE8	1.00±0.52	0.46±0.08	40.72±12.74	not detectable
	NE9	6.19±0.30	5.88±0.66	6.51±3.90	12.26±1.42
	NE10	0.50±0.54	0.92±0.31	34.71±10.05	66.67±47.14
	NE11	7.86±0.91	6.29±2.77	12.11±1.08	9.40±2.19
	NE12	5.72±3.77	5.52±1.07	10.24±2.00	13.65±2.92
	NE13	1.49±0.54	0.68±0.10	53.92±10.03	70.94±34.79
	NE14	1.03±0.81	0.84±0.36	29.69±3.25	not detectable
	NE15	0.82±0.85	0.64±0.26	48.12±11.37	47.22±3.93
	NE16	0.64±0.49	0.56±0.25	67.00±29.50	110.58±20.40
	Average	3.36±2.77	3.15±2.81	28.60±23.42	31.98±36.29
Central	C1	5.40±0.18	1.68±1.44	9.23±6.64	22.18±2.17
	C2	6.89±0.65	6.37±0.97	11.14±2.39	9.88±1.33
	C3	6.98±1.34	5.25±0.48	6.78±2.69	15.05±3.50
	C4	7.05±0.38	5.16±1.72	6.88±4.28	9.58±2.45
	C5	6.56±0.07	6.72±1.53	7.73±3.07	10.3±1.87
	C6	8.01±1.15	5.54±1.44	7.16±5.74	15.61±12.12
	C7	6.57±0.39	10.23±4.99	5.57±.084	10.35±2.03
	C8	6.41±0.60	5.36±1.15	9.86±2.99	10.28±5.51
	Average	6.74±0.931	5.79±2.35	8.04±1.86	12.91±6.02
West	W1	5.04±0.48	7.37±0.51	9.17±0.70	9.04±1.07
	W2	4.68±0.70	5.16±1.55	8.89±3.56	8.89±1.22
	W3	6.64±1.18	3.93±1.03	19.45±5.27	21.79±4.48
	Average	5.46±1.16	5.49±1.79	12.50±6.11	13.24±6.84
South	S1	5.73±0.90	5.16±2.06	8.75±3.00	13.67±2.57
	S2	8.45±0.53	6.05±0.76	7.19±4.90	12.84±1.50
	S3	7.14±0.59	8.09±3.71	13.08±5.32	14.27±3.98
	S4	3.31±0.38	5.37±1.42	5.60±1.86	18.19±9.37
	S5	2.60±0.33	2.46±1.02	13.83±5.31	18.11±10.32
	S6	7.07±0.45	8.09±3.71	5.47±2.29	11.05±2.31
	S7	6.97±0.48	7.54±0.89	15.18±3.45	12.89±1.79
	S8	6.18±0.85	5.08±1.12	6.73±3.63	15.87±4.40

Table 1 (continued)

Location	Code	Total curcuminoids (% w/w)		EC_{50} (mg/ml)	
		Before planting	After planting	Before planting	After planting
	S9	4.04±0.46	3.94±0.86	19.56±6.47	16.21±2.39
	S10	5.68±0.37	4.03±0.58	10.06±2.81	23.52±7.46
	S11	6.14±0.39	3.91±0.51	9.19±4.39	17.34±1.60
	S12	4.58±0.54	3.92±0.29	10.94±8.67	21.24±2.35
	S13	4.32±0.42	8.53±0.63	16.38±7.57	14.50±4.10
	S14	9.19±0.32	5.86±0.53	10.74±7.12	13.31±4.22
	S15	7.70±0.88	6.62±1.44	17.22±10.71	20.52±5.91
South	S16	5.51±0.37	5.38±0.43	7.19±1.64	13.70±0.52
	S17	5.39±1.20	2.67±0.78	9.71±7.89	21.24±2.08
	S18	6.55±1.13	5.29±0.90	9.62±2.89	12.84±2.55
	S19	4.44±0.26	6.16±0.58	8.76±4.36	5.60±1.32
	S20	5.68±0.84	12.39±0.73	5.26±3.58	13.46±13.13
	S21	5.06±0.34	3.49±0.44	8.84±1.09	16.51±2.88
	S22	4.46±0.26	3.69±0.44	9.17±4.55	28.23±7.46
	S23	5.27±0.42	4.65±0.42	8.94±3.99	15.32±6.31
	S24	4.69±0.49	6.90±2.85	7.92±1.59	7.63±1.84
	S25	7.53±1.49	0.58±0.49	11.44±3.90	50.75±12.52
	S26	7.36±1.20	1.82±0.88	10.21±2.94	18.85±4.10
	S27	7.85±1.16	3.06±1.11	11.43±1.04	18.54±4.17
	S28	9.84±0.93	6.12±0.95	10.51±3.37	13.50±0.91
	S29	10.13±1.27	2.72±0.96	6.74±2.41	11.07±1.16
	average	6.17±1.95	5.06±2.53	10.20±5.29	16.92±9.08
Lao PDR	Laos	1.09±0.35	1.20±0.38	12.88±4.29	33.36±3.56
C. mangga	Out	0.11±0.05	0.05±0.03	not detectable	not detectable

month-old rhizomes. However, the six-month-old rhizomes contained lower total curcumonoids than those found in the samples before planting (Table 2) because the original sources of the samples may have be older than six months, resulting in higher accumulative curcuminoids in the rhizomes.

These results are similar to Chavalittumrong and Jirawattanapon (1992), who studied variation in the active constituents of *C. domestica* rhizomes collected from Nakhon Pathom, Central Thailand, where they found the highest curcuminoids content was 10.12% w/w. In addition, Pothitirat and Gristsanapan (2006) reported that a sample from the South contained the highest total curcuminoids (8.99±0.83 %w/w), while the lowest was found in the North (4.80±1.83% w/w) where the climate is cooler and the dry period is longer and more pronounced.

Antioxidant activity

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration. Antioxidant activity using DPPH radical-scavenging assay reported with EC_{50} value is shown in Table 3. The lower the EC_{50} is, the higher the antioxidant activity of the compound. The antioxidant activity differed significantly in samples from different locations. The original samples collected from the central region showed the strongest antioxidant activity (EC_{50} value= 8.04±3.77 mg/ml), which was close to ascorbic acid (7.05±1.05 mg/ml). The six-month-old rhizomes exhibited lower antioxidant activity. A similar method using ethanol extraction of turmeric was investigated for its *in vitro* antioxidant activity using DPPH radical-

Table 2 Total curcuminoids content of the turmeric collections (by region) before planting and six months after planting.

Locations	Total curcuminoids (% w/w)	
	Before planting	6 months after planting
North(N=10)	4.94 ± 1.84bcA	4.18 ± 1.99bcA
Northeast(N=16)	3.26 ± 2.77bA	3.15 ± 2.18abB
Central(N=8)	6.74 ± 0.93cA	5.79 ± 2.87cA
West(N=3)	5.45 ± 1.16cA	5.49 ± 1.79cA
South(N=29)	6.17 ± 1.95cA	5.06 ± 2.53bcA
Lao PDR(N=1)	1.09 ± 0.35aA	1.20 ± 0.38aA
C. mangga	0.11 ± 0.05aA	0.05 ± 0.06aA
F-test	*	*
% CV	46.67	59.56

Means within each column followed by the same lower case letter are not significantly different at 95% level according to the Duncan's new multiple range test.

Means within each row followed by the same upper case letter are not significantly different at 95% level according to paired-samples testing.

Table 3 EC_{50} value of turmeric collections (by region) before and six months after planting.

Locations	EC_{50}(mg/ml)	
	Before planting	6 months after planting
North(N=10)	11.35 ± 5.61aA	16.30 ± 5.82abB
Northeast(N=16)	28.60 ± 23.42bA	31.98 ± 36.29bA
Central(N=8)	8.04 ± 3.77aA	12.91 ± 6.02aB
West(N=3)	12.50 ± 6.11aA	13.24 ± 6.84aA
South(N=29)	10.20 ± 5.29aA	16.92 ± 9.08abB
Lao PDR	12.88 ± 4.29aA	33.36 ± 3.56bB
C. mangga	not detectable	not detectable
F-test	*	*
% CV	69.20	69.68

Means within each column followed by the same lower case letter are not significantly different at 95% level according to the Duncan's new multiple range test.

Means within each row followed by the same upper case letter are not significantly different at 95% level according to paired-samples testing.

Ascorbic acid =7.05±1.05 mg/ml.

scavenging assay by Ungphaiboon *et al.* (2005) and showed stronger activity (EC_{50} = 11.26 µg/ml) than in this study. In addition, methanol extracts of Zingiberaceae have been assessed for free-radical scavenging activity against the DPPH radical and turmeric showed the strongest antioxidant activity with an EC_{50} value of 9.7 µg/ml. Consequently, it showed stronger antioxidant activity than in the results from the current study.

Comparisons of EC_{50} values of turmeric before and after planting were significant. The antioxidant activity and total curcuminoids content in six-month-old samples decreased when grown in the same environment.

The positive correlation between total curcuminoids content and antioxidant activity ($r = 0.582$, $p < 0.05$) showed that the antioxidant activity in turmeric was due to the presence of total

curcuminoids content. The same relationship was also observed between curcumin and antioxidant activity (Kapoor and Priyadarsini, 2001).

A previous study of active compounds of turmeric showed variability within *C. domestica* (Ratnambal, 1986).

Plant genotype, field conditions and postharvest processing can alter the combinations of phytochemicals in the turmeric rhizome (Cousins *et al*., 2007). In this study, turmeric samples were originally collected from different locations and planted in the same environment and yet they still exhibited variations in curcuminoids content and antioxidant activity. The results suggest that within-species genetic variations do exist in *C. longa* L. even though this plant species is clonally propagated.

Genetic diversity using RAPD technique

A total of 19 random primers was selected for RAPD analysis. The number of bands produced by each primer, the number of polymorphic bands and the percentage of polymorphism produced by each primer are presented in Table 4.

Nineteen random primers produced 184 scorable bands out of which 166 were polymorphic. The percentage of polymorphism ranged from a maximum of 100% for six random primers to a minimum of 66.67% by OPA 15. Nayak *et al*. (2006) demonstrated the utility of using RAPD markers to characterize genetic diversity among 17 promising cultivars of *C. longa*. Differential polymorphism was noted in 17 cultivars of turmeric showing variation in the percentage of polymorphic bands from 35.6% in PTS51 to 98.6% in Acc31 using 20 primers. The high number of polymorphic loci revealed profound intraspecific variation among the turmeric cultivars. Significant genetic

Table 4 Number of bands generated by each primer, number of polymorphic bands and percentage of polymorphism of turmeric collections.

No.	Primer	Total no. of bands	No. of polymorphic bands	Polymorphism (%)
1	OPA2	13	12	92.31
2	OPA3	11	9	81.82
3	OPA4	13	13	100.00
4	OPA8	6	5	83.33
5	OPA9	9	8	88.89
6	OPA15	9	6	66.67
7	OPA18	8	7	87.50
8	OPJ9	10	9	90.00
9	OPR1	11	9	81.82
10	OPR2	12	10	83.33
11	OPR7	11	11	100.00
12	OPR8	8	8	100.00
13	OPR13	10	9	90.00
14	OPR15	6	5	83.33
15	OPS11	9	8	88.89
16	OPS12	10	10	100.00
17	OPT5	9	8	88.89
18	OPT8	11	11	100.00
19	OPT20	8	8	100.00
Total		184	166	89.83

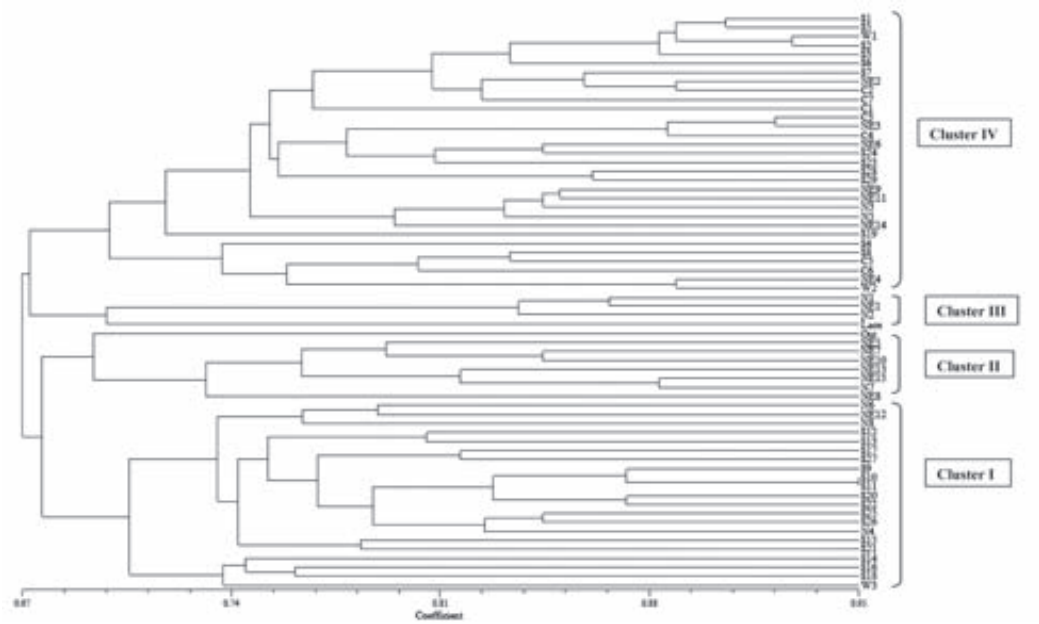

Figure 2 Dendrogram of turmeric from a UPGMA cluster analysis obtained from 19 RAPD primers.

variations by RAPD markers have also been reported in other species at cultivar level (Colombo *et al.,* 1998; Das *et al.,* 1998; Huang *et al.,* 2003).

Cluster analysis

The dendrogram based on the UPGMA clustering of the RAPDs shows the genetic similarity of all accessions (Figure 2). At 68.4% similarity, all samples were separated into four clusters. The accessions of turmeric populations were mixed together and placed in several clusters. Cluster I contained 21 accessions of most samples from the South, except N4, N6, N8, NE12 and W3. Cluster II consisted of six turmeric samples from the Northeast, a collection of *Curcuma mangga* and N7. Moreover, Cluster III had four accessions, with the accession from Lao PDR showing 70% similarity with N1, N2 and NE1. Cluster IV consisted of a mix from all collections; 7 collections from Central, 2 collections from North, 7 collections from Northeast, 13 collections from South, and 2 collections from West. The results from cluster analysis did not show distinct clusters by region. The mix of collections from different regions in a cluster might have been due to the movement of planting material throughout the country.

Islam *et al.* (2007) reported a high level of genetic diversity within *C. zedoaria* populations. Hamrick and Loveless (1989) also reported a common trend in tropical plants of maintaining high genetic diversity within populations. Paisooksantivatana *et al.* (2001) investigated the genetic diversity of *C. alismatifolia* Gagnep. in Thailand using allozyme polymorphism and found high levels of genetic diversity within a population. In addition, the overall genetic diversity of all populations of *C. alismatifolia* was high possibly due to a wide range of ecological conditions within the distribution area of its populations in Thailand.

Principal component analysis (PCA)

Figure 3 shows a 3-dimentional PCA plot of turmeric RAPD data. The accessions plotted

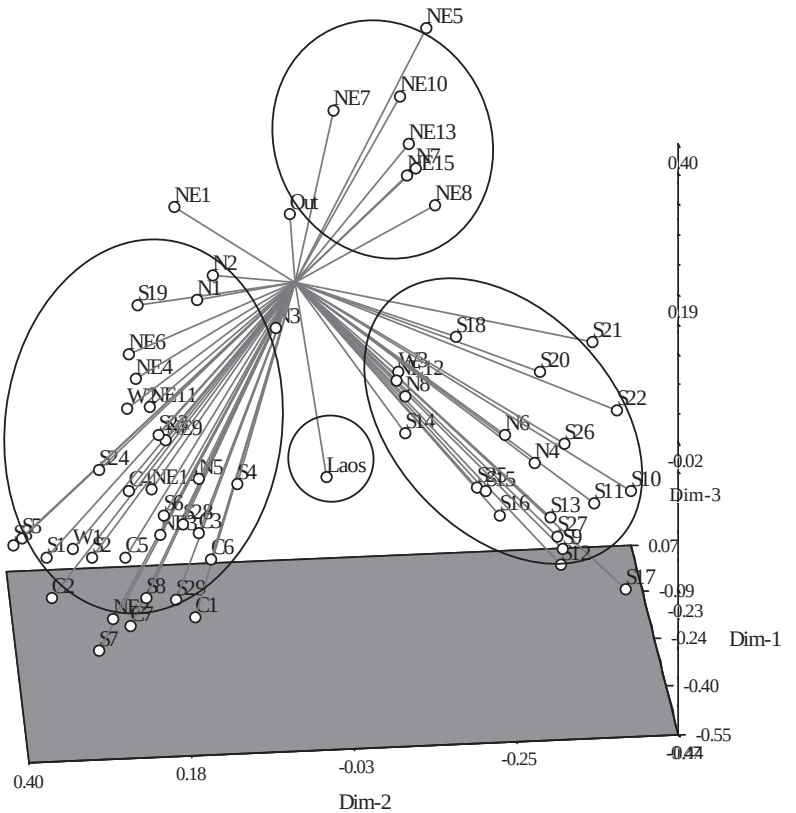

Figure 3 Principal component analysis of 64 samples of *Curcuma longa* L. collected from different locations.

are labeled according to their informal grouping. It clearly distinguishes four groups of turmeric, similar to the dendrogram based on the UPGMA clustering.

CONCLUSIONS

The highest total curcuminoids content was found in the samples from the central region while the lowest content was found in the samples from the Lao PDR. Total curcuminoids content decreased after planting for six months.

The highest antioxidant activity (EC_{50} 8.04±3.77 mg/ml) was found in the samples from the central region and antioxidant activity decreased after planting for six months.

The RAPD technique using the UPGMA algorithm and PCA revealed high diversity within and among clusters.

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