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Genotypic diversity of turmeric (*Curcuma longa* L.) accessions in mindanao, philippines on the basis of curcumin content

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Abstract

Twenty-one turmeric accessions collected from seven provinces in Mindanao were grown in a uniform environment and analyzed for curcumin content. Rhizome bits of each accession were planted in big plastic bags and randomly laid out in the field. Rhizome samples harvested from each accession were analyzed for curcumin content using High Performance Liquid Chromatography (HPLC) on a Novapak C_{18} column with the solvent system consisting of methanol:acetonitrile (80:20) and detection at 370 nm. Highly significant differences in curcumin content were observed among the turmeric accessions, varying from as low as 1.2 mg/g for Accession 3 to as high as 102.4 mg/g for Accession 20. These results reveal the existence of genetic variation in the turmeric accessions collected from the different provinces in Mindanao with high curcumin yielding accessions identified which could be recommended for cultivation.

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Introduction

Turmeric (*Curcuma longa* L.) is a tropical perennial herb belonging to family Zingiberaceae. It was originally valued mainly as a spice for food and natural dye for clothing until recently when it was discovered as a potential source of new drugs for a variety of diseases. Its importance in medicine started with the discovery that the dried rhizome of the plant is rich in phenolics, identified as curcuminoids, particularly curcumin or diferuloyl methane. Some of the biological activities and therapeutic properties attributed to curcumin were anti-inflammatory, anti-oxidant, anti-carcinogenic, wound healing, and anti-viral properties (Joe *et al*., 2004).

Turmeric is a cross-pollinated, sterile, triploid species that is clonally propagated using its underground rhizomes. Though vegetative propagation is the usual means of reproduction, several studies have shown the existence of genetic variation in the species. Nayak *et al.* (2006) was able to detect genetic variation among 17 promising cultivars of turmeric using 4C nuclear DNA content and random amplified polymorphic DNA (RAPD) analysis. Similarly, Leong-Skornickova *et al.* (2007) observed more than 9% genome size variation in two accessions of *C. longa* having the same chromosome number. According to the authors, the observed intra-specific variation may be related to the long-term cultivation and targeted selection of desirable genotypes in *C. longa*, which in turn may have adaptive value to the crop. A manifestation of the observed intra-specific variation is the probable varied potential of different genotypes to synthesize curcumin. Analyzing several studies, Sasikumar (2005) reported that total curcumin may vary from 2-7% with the turmeric cultivars classified either as high or low curcumin varieties.

Turmeric is currently cultivated in different provinces in Mindanao and is processed mainly as tea granules and capsules both of which are widely used by practitioners of alternative medicine. However, cultivation of turmeric in these areas seldom take into consideration the potential of the variety used as planting material to synthesize curcumin. In the Philippines where turmeric is reported to be an introduced species, very little study has been conducted to assess the genetic diversity which may exist due to the varied introduction of turmeric from different sources. No study has yet been conducted to verify if there are significant differences in the curcumin yield of the varieties being cultivated. This study therefore aims to determine the possible existence of variation in curcumin content among different turmeric accessions collected from different provinces in Mindanao which may also be an indication of genetic variation among these accessions. It also aims to identify potential high yielding accessions which could be recommended for cultivation in the different areas of Mindanao.

Materials and methods

Collection of turmeric rhizome

Turmeric rhizomes were obtained from different localities in seven provinces in Mindanao, Philippines, namely, Davao del Sur, Davao del Norte, South Cotabato, North Cotabato, Lanao del Sur, Sultan Kudarat and Maguindanao. These were designated as turmeric accessions 1-17 and 19-22. Fig. 1 shows the different provinces from where each accession (Acc) was collected. For comparison, turmeric rhizome sample obtained from Chiang Mai, Thailand was designated as turmeric accession 18. Collected rhizomes of each accession were washed clean of debris and air-dried for two days. Air-dried rhizome bits/finger rhizomes were then used as planting materials.

Maintenance of turmeric accessions in the field

The different turmeric accessions were planted in a 500 m^2 area. Rhizome bits of each accession were initially planted in big plastic pots (10 rhizome bits/bag) containing garden soil mix. Germinated buds were individually replanted in small plastic bags containing the same garden soil mix. Ten plants of each accession were replanted in big plastic bags (24 in x 36 in) containing bagging media composed of 70% coconut coir and 30 % soil, and then randomly

laid out in the field. All plants were watered daily except when heavy rain occurred the previous night or during the day. Monthly fertilization using complete fertilizer or ammonium sulfate was done. Vermicast incorporated with *Trichoderma* was added a month after replanting in big plastic bags. Spraying of insecticide (Decis) was done whenever insect infestation was observed. Rhizomes of each accession were harvested when the lower leaves of the plants started to wither, about 7-8 months after planting, depending on the accession.

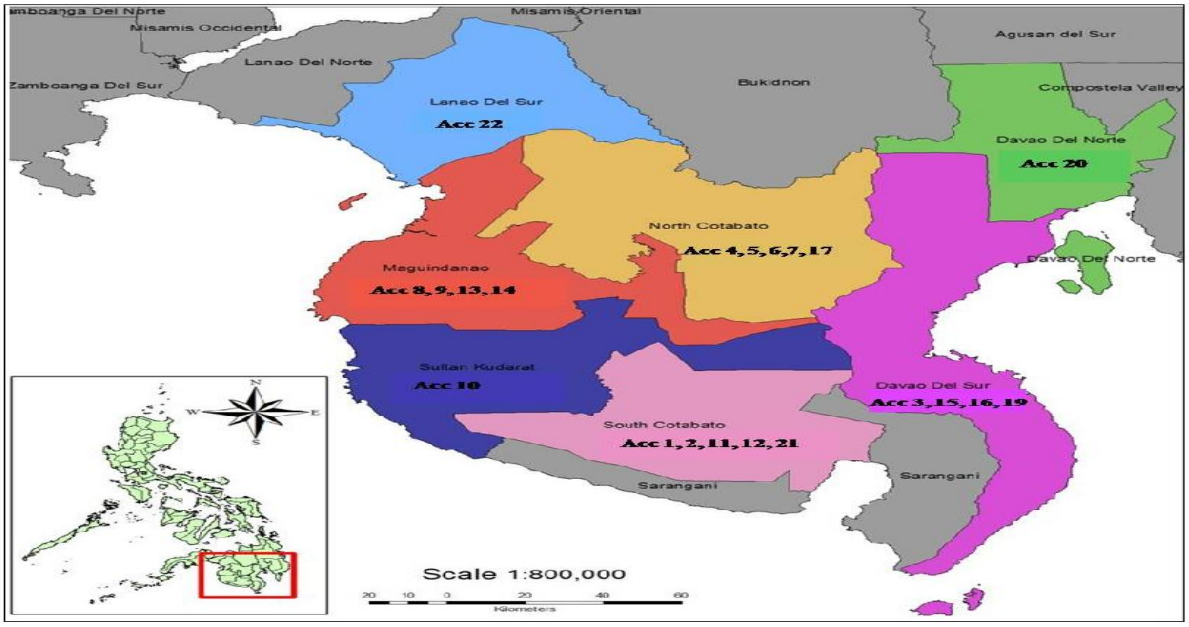

Fig. 1. Map of Mindanao showing the provinces where the turmeric rhizomes were collected.

Preparation of rhizomes

Rhizomes of each accession were washed separately with soap and water and allowed to air dry. Air-dried rhizomes were sliced thinly and oven-dried in a convection oven (Binder) set at 40°C for two days. Approximately 50 g of the oven-dried samples of each accession were packed in plastic bags and sealed with a plastic sealer.

Determination of moisture content

The moisture content of the oven-dried rhizome samples of each accession was determined prior to analysis for curcumin content. Approximately 5 g sample was placed in a small envelope and dried to constant weight in a forced draft oven set at 70°C. Moisture content (MC) was computed using the formula:

$$\%MC = \frac{W_i - W_f}{W_i} \times 100$$

Where:

W_i = weight of sample prior to drying

W_f = weight of sample after drying.

The moisture content of the rhizomes of each accession at harvest was also determined using the same procedure. Moisture content data was used to estimate the dry weight of the rhizomes at harvest, which in turn was used in computing the curcumin yield of each accession on a dry weight basis.

Thin layer chromatography (TLC) of turmeric rhizome extracts

Methanol extracts of the oven-dried rhizome of the different accessions were subjected to TLC to determine if they are truly *C. longa* instead of the morphologically similar species *C. xanthorhiza*. Methanol extraction of curcuminoids was done by immersing approximately 1 g of rhizome in 5 mL of methanol for 5 min in a water bath maintained at 60°C. The solvent system used for TLC was chloroform:ethanol:glacial acetic acid (95:5:1). Presence of the three distinct yellow bands in the

chromatogram, corresponding to the three curcuminoids, distinguishes *C. longa* from *C. xanthorhiza*, which has only two bands (Wagner and Bladt, 1996).

Determination of rhizome curcumin content using HPLC

The procedure followed for curcumin analysis was based on the method developed by Jayaprashka *et al.* (2002) and modified for the analysis of curcumin content only since this is the most important active ingredient responsible for the biological activity of turmeric.

One (1.0) g of ground turmeric sample of each accession was extracted with 200 mL of N-hexane using a Soxhlet extractor for 30 min. The hexane extract was discarded and the sample was further re-extracted with 200 mL of methanol for two hours. Duplicate extractions were done for each sample accession. A 1-mL aliquot of the methanolic extract transferred to a 10-mL volumetric flask and diluted to the mark with methanol was used for HPLC injection. For the analytical standard, a known amount of curcumin (98% purity; Fluka cat # 0851.1) was weighed in a 10-mL volumetric flask, dissolved and diluted to the mark with methanol to arrive at a 1000 ppm stock solution. Aliquots were taken from the stock solution to prepare the working standard solutions needed to establish the standard curve.

Twenty microliters (20 µL) of each of the samples and the working standard solutions (containing active ingredient ranging from 0.0078 µg to 1.9600 µg) was injected into Shimadzu Liquid Chromatograph 10AT. The chromatographic column used was a Novapak C18, 3.9 x 150 mm, 4 µm column and detection was done using Shimadzu SPD-10AV-UV detector at 370 nm wavelength. Elution was carried out with a solvent system consisting of methanol:acetonitrile (80:20 v/v mixture) at a flow rate of 0.8 mL min^{-1}.

The curcumin content of the sample extracts were calculated based on the standard curve established using the formula:

$y = b + mx$

Where:

y = peak area of the injected sample

x = amount of active ingredient in µg

b = y-intercept of the curve

m = slope of the curve.

Curcumin content of the rhizome samples of each accession were expressed as % curcumin (w/w) dry weight basis. Data on curcumin content was subjected to one-way ANOVA to determine if there were significant differences among the accessions.

Results

TLC of turmeric rhizome methanol extracts

The chromatogram of the rhizome methanol extracts of all the turmeric accessions showed the presence of the three curcuminoid pigments: curcumin, demethoxycurcumin and bisdemethoxycurcumin (Fig. 2). The presence of the band corresponding to bisdemethoxycurcumin in all of the accessions confirms that all belong to the species *C. longa* instead of the close relative *C. xanthorrhiza*. Nevertheless, variation in the intensity of the different curcuminoid bands, particularly curcumin, among the turmeric accessions was observed. Acc 2, 13, 14, 19, 20, 22 showed very intense bands, whereas Acc 3 showed very faint bands for all three curcuminoids. The observed variation in the intensity of the three curcuminoid bands was already an indication of the possible variation in the curcumin content among the rhizome samples of the turmeric accessions.

HPLC determination of turmeric rhizome curcumin content

Table 1 shows the result of the analysis for curcumin content of the 22 turmeric accessions. Results revealed highly significant differences in the curcumin content of the accessions. Acc 20 yielded the highest curcumin content (102.4 mg/g dry weight) while Acc 3 gave the lowest (1.2 mg/g dry weight). Expressed in terms of percent curcumin, the

range would be from 0.12 10.24%.

Curcumin yield of the various turmeric accessions

Computation of the curcumin yield of each plant based on estimated dry weight at harvest is presented in Table 2. Acc 16 gave the highest curcumin yield of 8093 mg among all the turmeric accessions. Though the percentage curcumin content (38 mg/g) recorded for this accession was more or less intermediate among the accessions, the relatively low moisture content (0.77) of the rhizomes resulted to a relatively high estimated dry weight (213 g) which compensated for the not so high curcumin content. The next highest curcumin yield was recorded for Acc 20, 13 and 22 which was mainly due to the high percentage curcumin content recorded for these accessions. Acc 6 and 7 also gave comparable curcumin yields which were also due to a relatively high estimated dry weight and intermediate curcumin content. The lowest curcumin yield was again recorded for Acc 3 due to the very low curcumin content as well as low rhizome yield.

Table 1. Average curcumin content of the 22 turmeric accessions.

Accession Number	Rank	Curcumin Content (mg/g) (MG/G)
1	4	15.3 ^{def}
2	13	21.4 ^{def}
3	1	1.2 ^{e}
4	7	17.9 ^{def}
5	10	19.6 ^{def}
6	16	27.7^{def}
7	15	27.5^{def}
8	3	9.8 ^{ef}
9	12	21.3^{def}
10	14	24.2 ^{def}
11	17	30.2^{cde}
12	9	19.3^{def}
13	21	72.8 ^{b}
14	19	39.0 ^{cd}
15	11	21.0^{def}
16	18	38.0 ^{cd}
17	5	15.7 ^{def}
18	8	19.2^{def}
19	6	17.1 ^{def}
20	22	102.4 ^{a}
21	2	6.3 ^{ef}
22	20	52.8 ^{bc}

Means with the same letters are not significantly different at Tukey HSD 0.05

Ranking is from lowest to highest curcumin content.

Discussion

Several studies have already reported the existence of variation in curcumin content among different turmeric accessions. Variation was exhibited not only among the different genotypes evaluated but also with the range of curcumin content reported. The study of Garg *et al.* (1999) resulted to a 0.61 to 1.45% range in curcumin content among the 27 accessions evaluated while Pandey and Katiyar (2010) reported curcumin content to vary from 0.15 to 1.87% in the 22 genotypes analyzed. Jayaprashka *et al.* (2002) obtained a range of 1.06 to 5.65% curcumin in four commercially available varieties of turmeric. On the other hand, a range of 0.3 to 3.24% curcumin was obtained by Pathak *et al.* (2010) in turmeric samples from different zones in India, while Kulkarni *et al.*

(2012) reported a curcumin yield of 12.39% from turmeric samples collected from a Satara district in India. A comparison of the curcumin content of 'ISSR Kedaram', a high yielding variety released in India, and its open-pollinated progenies showed that the mother yielded a significantly higher curucmin content of 5.67% (Nair *et al.*, 2010).

Table 2. Curcumin yield of the 22 turmeric accessions.

Accession Number	Fresh Weight (g)	Moisture Content (%)	Dry Weight (g)	Cucumin Content (mg/g)	Curcumin Yield (mg)
1	711.3	84	113.8	15.3	1741
2	579.3	86	81.1	21.4	1736
3	394.7	66	134.1	1.2	161
4	1018	83	173.1	17.9	3098
5	849.3	84	135.9	19.6	2663
6	1132.3	83	192.5	27.7	5332
7	899.7	79	188.9	27.5	5196
8	1036.7	82	186.6	9.8	1829
9	1058.3	80	211.7	21.3	4508
10	742.7	76	178.2	24.2	4314
11	266.3	84	42.6	30.2	1287
12	833.7	84	133.4	19.3	2574
13	638.3	87	83.0	72.8	6041
14	528.7	86	74.0	39.0	2887
15	776	82	139.7	21.0	2933
16	926	77	213	38.0	8093
17	1042.7	78	229.4	15.7	3601
18	1022	83	173.7	19.2	3336
19	620.3	83	105.4	17.1	1803
20	540	88	64.8	102.4	6636
21	932.7	83	158.6	6.3	999
22	799.3	86	111.9	52.8	5908

Different methods for the quantification of curcumin were used in the mentioned studies. The study of Pathak *et al.* (2010) and Nair *et al.* (2010) made use of spectrophotometric methods while high performance thin layer chromatographic method (HPTLC) was used by Garg *et al.* (1999) in the estimation of curcumin. On the other hand, the studies of Jayaprashka *et al.* (2002), Pandey and Katiyar (2010) and Kulkarni *et al.* (2012) analyzed the curcumin content using HPLC although the chromatographic conditions followed were also different. Variation in the method used in estimation/quantification may also be one factor contributory to the differences in the reported values for curcumin content.

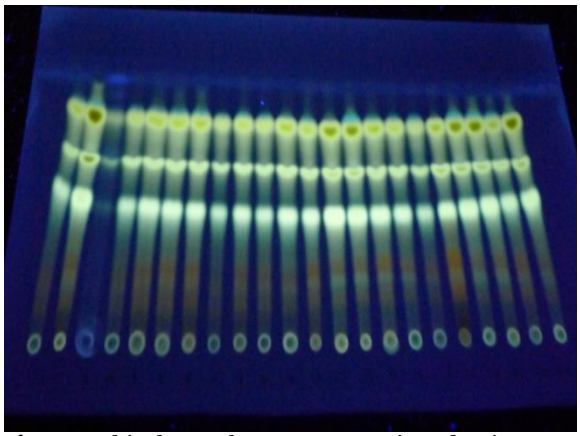

Fig. 2. Thin layer chromatogram viewed using 365 nm UV light showing the presence of the three curcuminoid pigments (A: curcumin, Rf=0.6; B: demethoxycurcumin, Rf=0.5; C: bisdemethoxycurcumin, Rf=0.4) in the 22 turmeric accessions.

The present study made use of modified HPLC optimized for estimating the curcumin content present in the different turmeric accessions. Comparison of the curcumin content of the present turmeric accessions with those that have been reported shows that several of the accessions had curcumin contents that would be in the upper limit of the ranges reported. Among these are Acc 20 (10.24 %), Acc 13 (7.28%), Acc 22 (5.28%), Acc 14 (3.89%) and Acc 16 (3.80%). Acc 20, 13 and 22 even exceeded or equalled the curcumin content of 'ISSR Kedaram', a reportedly popular high yielding variety released in India (Nair *et al.* 2010). Present results, therefore, indicate the existence of potential high curcumin yielding genotypes in the present germplasm, which after consideration of other important characters such as yield, could be recommended for cultivation.

The significant differences in curcumin content observed may be an expression of genetic diversity among the turmeric accessions. Since all the accessions were planted in the same environment and subjected to the same growth conditions, any variation in the curcumin content could be attributed either to a possible difference in the genotypes of these accessions, particularly the genes coding for the enzymes in the biosynthetic pathway leading to the synthesis of curcumin and other curcuminoids, as well as the varied expression of these enzymes. Katsuyama *et al.* (2009a, 2009b) identified three curcumin synthase isoforms (CURS 1, CURS 2 and CURS 3) involved in the synthesis of the three major curcuminoids. These three enzymes, which have different substrate preferences, are polyketide synthases responsible for the hydrolysis of feruloyldiketide-CoA. CURS 1 converts feruloyldiketide-CoA esters into curcumin using feruloyl-CoA exclusively as starter substrate. CURS 2 preferred feruloyl-CoA as starter substrate and catalyzes the synthesis of curcumin and demethoxycurcumin, while CURS 3 preferred both feruloyl-CoA and p-coumaroyl-CoA as starter substrate and catalyzes the synthesis of curcumin, demethoxycurcumin and bisdemethoxycurcumin. It is presently hypothesized that the resulting composition of curcuminoids in turmeric and its different cultivars might be influenced by the availability of substrates as well as the expression levels of the three CURS enzymes that have different substrate specificities.

The observed variation in curcumin content among the turmeric accessions, which is an indication of genetic diversity among the accessions, revealed potential high curcumin yielding genotypes which could be recommended for cultivation to growers engaged in production and processing of different turmeric herbal preparations used in alterative medicine. However, the performance of these accessions may vary when these are grown in a different environment, since curcumin content has been reported to be influenced not only by the genotype but also by the environment as well as the possible interaction of the genotype and environment. Hence, cultivation of these accessions in different locations and environmental conditions may be necessary to better assess the potential of each accession in terms of rhizome yield and curcumin content.

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