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Research Article
Identifying Turmeric Varieties (*Curcuma* spp.) Based on their Morphologies and Molecular Markers

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Abstract

Background and Objective: Turmeric is an economically important spice crop in Asia. Among numerous turmeric accessions, some of them have high rhizome yield, high curcuminoids content, and well-adapted ability to diverged agro-climatic ecosystems. This study was carried out to evaluate the diversity of 34 turmeric samples based on their morphological, molecular and biochemical markers. **Materials and Methods:** A total 34 turmeric samples were taken (32 Vietnamese samples, one Indonesian sample, and one Australian sample). The morphological characteristics included the morphology of the trunk, leaf, flower and rhizome. The yield and curcumin content of collected *Curcuma* could be estimated based on morphological characteristics. The molecular markers included ten RAPD markers, ten ISSR markers and RAPD markers. **Results:** Thirty fours samples of local and imported turmeric were characterized based on morphological traits. The dendrogram was split into three groups (group I*: Curcuma zedoaria*; group II: *Curcuma aeruginosa*; group III: *Curcuma xanthorrhiza*, *Curcuma longa* and *Curcuma amada*) at a similarity coefficient of 0.68. The results illustrated that the evaluated accessions belonged to *Curcuma longa* L., *Curcuma* species. The highest curcumin content and yield of dry rhizome recorded in C.34 (*Curcuma xanthorrhiza*) were 12.4% and 11.6 g, respectively. **Conclusion:** With the high yield of curcumin and dry rhizome, C.34 variety could be cultivated for curcumin's purpose in the future.

Key words: Turmeric varieties, *Curcumin xanthorrhiza*, molecular markers, RAPD, ISSR

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus of turmeric (*Curcuma*) belongs to the ginger family Zingiberaceae and is an important medicinal herbs[1]. Most known turmeric varieties have been identified based on their morphological and biochemical differences[2]. While the polymorphisms of stem, leaf, flower and bulb are essential in identifying turmeric varieties, they are strongly affected by environmental conditions. In addition, these phenotypic traits have low polymorphism and require time and experience to employ. The combination of molecular techniques and agro-biological criteria has improved the effectiveness and accuracy of the selection and identification of turmeric varieties. To evaluate the genetic diversity of turmeric, RAPD markers[3-5] and ISSR markers[6] have been commonly used due to their ease, accuracy and simplicity of implementation. The benefits of curcumin have been reported as a polyphenol found in the rhizome of turmeric in anticancer mechanisms, anticancer, anti-oxidant, antiviral, anti-inflammatory, antiparasitic, antifungal and detoxification[7-10]. However, the curcumin content in turmeric is low and ranges from 1-6%[11]. Among different factors that affect the content of curcumin in turmeric, variety plays the most significant role[12-15]. Leong-Škornicková *et al.*[16] reported 27 species of turmeric that scattered from the North to the South of Vietnam. This would be a valuable source of genetic material to identify turmeric with desired traits. However, the number of research about turmeric varieties and nutrition is limited. Therefore, the evaluation of the curcumin content and yield, morphology and molecular and biochemical characteristics of turmeric would be implemented for identifying varieties with desired cultivation time and high yield. In addition, this study would provide information about the chemicals that affect the growth and development of turmeric as well as the content and yield of curcumin.

MATERIALS AND METHODS

Study area: The field experiment was carried out on the farm of the College of Agriculture, Can Tho University from April, 2021 to December, 2022. Morphological analysis was conducted at the Crop Science Lab of the Faculty or Crop Science, College of Agriculture. The DNA analysis were carried out at the Institute of Food and Biotechnology, Can Tho University, Vietnam.

Sample collection: Thirty-four samples of domestic and imported turmeric were grown in Binh Thuy District, Cantho City, Vietnam. The experiment was carried out in a Randomized Complete Block Design (RCBD), with three replications. The plot size was 2 m^2. The plant was grown on sand foundation mixed with soil to have good water drainage. Secondary rhizomes with uniform weight (30-40 g) were selected. Seed-rhizomes were treated with chlorine (0.5%) for 30 min, dried and incubated for one week before being cultivated. Rhizomes were incubated in the shade on a high foundation that had a sufficient water supply and was covered by a layer of straw ash.

After 7-10 days, rhizomes were planted at the depth of 7-8 cm. The spacing was 25×25 cm. Water was applied adequately every day to maintain optimum soil moisture level for proper seedling emergence and plant growth. Weed management was conducted by hand every two weeks.

Fertilization: At 60, 120 and 180 days after planting (DAP), NPK (16-16-8-13S) was applied at the rate of 100-150, 150-200 and 100-150 kg/ha, respectively. At 60 DAP, the plants reached V2-V3. At 120 DAP, the finger had initiated and shoot growth was maximized. At 180 DAP, rhizome growth dominated.

Morphological characteristics: The morphological characteristics such as leaf form, flowering, color of flower, form of rhizome, color of rhizome and other growth characteristics were recorded 200-210 days after planting when the plant had fully grown[17]. Rhizome compositions were recorded at harvesting (240 days after planting).

Molecular characteristics

Collecting leaf samples: Two to three young and healthy turmeric leaves of each turmeric variety were collected, wrapped in polyethylene, sealed, labeled and stored at 4°C until use.

Isolation of DNA

Isolation and purification of DNA: The DNA from the leaf tissue of 34 samples was isolated and purified using the modified CTAB protocol[18].

Checking DNA by agarose gel electrophoresis

PCR reaction: The PCR reaction for each DNA turmeric samples were reacted with markers of 20 primers RAPD (OPA02, OPA03, OPA04, OPA10, OPA13, OPB07, OPB10, OPD02, OPD03 and OPD07) and ISSR (ISSR1, ISSR2, ISSR5, ISSR6, ISSR7, ISSR10, ISSR12, ISSR14, ISSR17 and ISSR18).

Electrophoresis of PCR components: An electrophoresis was carried out for PCR components with 1% agarose gel in TAE 1X by an M36 HexaGel™ electrophoresis apparatus (Edvotek, USA) with 42V in 30 min and 60V in 65 min. Ethidium bromide (1 mg/L) was added and poured into the gel in 20 min, washed with water and used an E3000 UV transilluminator (Accuris, USA) to capture the gel. The appearance of bands were the products of PCR amplification which was shown on agarose gel to distinguish the diversification of turmeric species.

Biochemical characters: The analysis of biochemical characterization was to analyze quality characteristics in morphological characters.

Data analysis: The morphological data presented in this research are the mean values of three replications. All data were analyzed using One-way Analysis of Variance (ANOVA) using SPSS software package version 13.0 and were compared for significant differences in treatment effects using Duncan's test at $p<0.05$ or $p<0.01$.

RESULTS AND DISCUSSION

Morphological traits of 34 turmeric samples

Characteristics of pseudostem, leaf and flower: Results showed that there was an insignificant difference in the color of the leaf and spike position. There was a significant difference in leaf blade shape, the appearance of petioles, leaf habit, the color of the midrib, the color of pseudostem and the color of the bract. Most of the turmeric samples had green pseudostem (79.0%), leaf petiole (85.0%), lanceolate leaf blades (73.0%), straight leaf (63.0%) and green midrib (71.0%). All leaf samples had special aroma and flowering. Inferior bracts were green and the superior ones were light green/light pink/white which occupies high percentage (70%).

Morphological traits of rhizome: There was no significant difference in the shape and taste of the rhizome. The shape of the mother rhizome, rhizome color, color and taste of the rhizome were the significant differences. Most varieties have an oblong mother's shape (73.0%), pale yellow rhizome (53.0%) and a special smell of turmeric. Although there are different colors of turmeric rhizomes, most of them were yellow (35.0%) Fig. 1(a-f). The results were appropriate to other studies. According to Syamkumar and Sasikumar[19], most of the 15 surveyed turmeric samples had camphor aroma, bitterness, or quite bitter (100%). According to Syahid and Heryanto[20], in 12 varieties of white *Curcuma zedoaria*, most of them had white rhizome inner core (91.7%), except Curz10 which had yellow rhizome.

Quality characteristics: The samples of C.34 (11.6 g), C.11 (11.2 g), C.13 (11.3 g) and C.18 (11.2 g) had the highest yield of curcumin (Table 1). The results were appropriate to the studies of other authors. Thaikert and Paisooksantivatana[21] reported that the curcumin content of 67 Thailand turmeric varieties ranged from 0.32±0.44 to 10.13±1.27%. The content of curcumin was one of three important biochemical components in *Curcuma* spp.[17]; content of curcumin in *C. longa* (2-5%) was higher than others. The content of curcumin was one of the components which decided the yield of curcumin[22].

Relationship between growth and quality characteristics: Correlation between the weight of fresh rhizome and dry rhizome/clump was statistically significant ($r = 0.96^{**}$). In addition, the weight of fresh rhizome and dry rhizome/clump had a significantly positive linear correlation according to ($y = 0.29x-3.89$, $R^2 = 0.87$) (Fig. 2). Regression model used the weight of fresh rhizome to estimate the weight of dry rhizome was appropriate and had statistical significance. The linear regression model explained the 87% difference in dry weight of rhizome/clump among samples.

The correlation between yield and content of curcumin on the weight of dry rhizome was significant ($r = 0.93^{**}$). In addition, the yield and content of curcumin on the weight of dry rhizome had a linear relation according to equation $y = 0.87x+0.15$; in which $R^2 = 0.86$ (Fig. 3) with $p<0.001$. The regression model used the content of curcumin to estimate the yield of curcumin was appropriate and had statistical significance. The linear regression model explained the 86% difference in dry weight of rhizome/clump among samples.

Relationship between quality characteristics and morphological characters

Relationship between the content of curcumin and morphological characters: Samples having a low content of curcumin (0.68±0.52%) had a light purple colored leaf midrib; white or pale yellow rhizome or dark purple inner and pale purple outer, camphor aroma, bitter taste; dark purple bract or green inferior and reddish purple superior. The characteristics of the red-brown midrib, dark purple rhizome, pale purple outer, reddish-purple superior bract; was

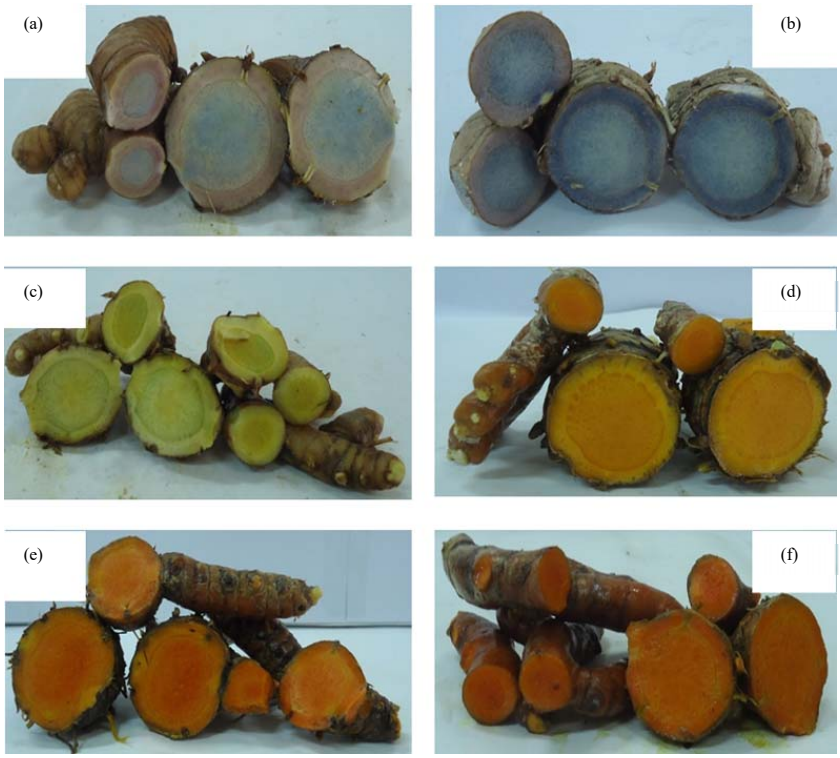

Fig. 1(a-f): Rhizome colour variation in the *Curcuma* species (Can Tho, Vietnam), (a) Pale blue, (b) Blue black, (c) Pale yellow with bluish green outer ring, (d) Pale yellow and (e-f) Orange yellow

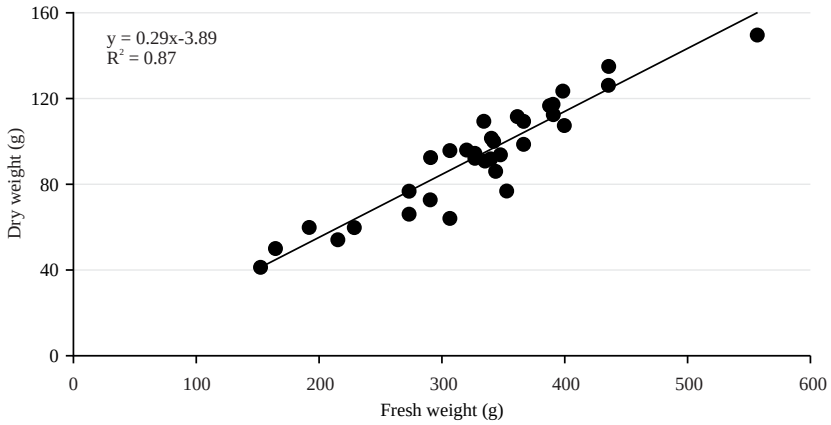

Fig. 2: Relationship between weight of fresh and dry rhizome/clump of 34 samples of local and imported turmeric

anthocyanin; the synthesis of curcumin (yellow) was not given priority so the content of curcumin of these samples was low. According to Lim[23], these samples belonged to *Curcuma aeruginosa* and *Curcuma zedoaria*.

Samples with an average content of curcumin (6.09±1.38%) had green midrib, yellow flesh, turmeric aroma, a little bitter and less methylated. The color of the superior bract was light green/light and pink/white. With the above morphological characters, according to Lim[23], these samples might belong to *Curcuma aromatica* and *Curcuma mangga*.

Samples with a high content of curcumin (10.5±1.11%) had green midrib, yellow or light orange yellow or dark yellow flesh, turmeric aroma, a little bitter and less methylated or no taste. The color of the superior bract was light green/light pink/white. According to Lim[23], these samples might belong to *Curcuma longa* and *Curcuma xanthorrhiza*.

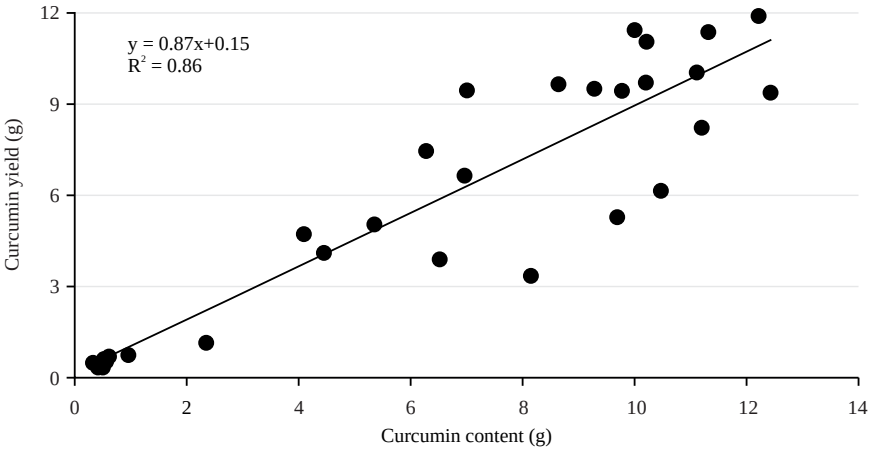

Fig. 3: Relationship between yield and content of curcumin/weight of dry rhizome of 34 samples of local and imported turmeric

Table 1: Quality traits of 34 samples of local and imported turmeric

Sample name	Weight of fresh rhizome (g)	Weight of dry rhizome (g)	Curcumin content (%)	Curcumin yield (g)
C.1	192^{fgh}	59.6^{ghij}	6.51^{m}	3.89^{gh}
C.2	228^{cdefgh}	59.4^{ghij}	10.5^{e}	6.22^{defg}
C.3	366^{bcd}	110^{abcde}	8.62^{j}	9.43^{abc}
C.4	215^{efgh}	53.8^{hij}	9.68^{h}	5.21^{efgh}
C.5	291^{bcdefgh}	72.8^{efghij}	11.2^{cd}	8.16^{bcd}
C.6	391^{bc}	117^{abcd}	9.99^{g}	11.30^{a}
C.7	274^{cdefgh}	76.6^{defghij}	0.96^{s}	0.75^{i}
C.8	152^{h}	41.0^{j}	8.14^{k}	3.35^{h}
C.9	436^{ab}	135^{ab}	6.99^{l}	9.44^{abc}
C.10	342^{bcde}	99.3^{bcdefg}	0.42^{uv}	0.42^{i}
C.11	366^{bcd}	110^{abcde}	10.2^{f}	10.30^{ab}
C.12	291^{bcdefgh}	93.1^{bcdefgh}	4.42^{p}	4.13^{gh}
C.13	390^{bc}	113^{abcde}	6.27^{n}	7.36^{cde}
C.14	306^{bcdefg}	64.3^{fghij}	5.32^{o}	3.42^{fgh}
C.15	334^{bcdef}	110^{abcde}	0.63^{t}	0.68^{i}
C.16	436^{ab}	126^{abc}	0.53^{tu}	0.68^{i}
C.17	341^{bcde}	102^{bcdef}	9.28^{i}	9.51^{abc}
C.18	366^{bcd}	98.7^{bcdefg}	11.3^{c}	11.20^{a}
C.19	327^{bcdef}	94.9^{bcdefgh}	6.96^{l}	6.62^{def}
C.20	353^{bcde}	76.5^{defghij}	12.4^{a}	9.50^{abc}
C.21	347^{bcde}	93.6^{bcdefgh}	10.2^{f}	9.56^{abc}
C.22	341^{bcde}	92.0^{cdefgh}	0.56^{tu}	0.53^{i}
C.23	362^{bcde}	112^{abcde}	0.63^{t}	0.69^{i}
C.24	398^{bc}	123^{abc}	0.52^{tu}	0.64^{i}
C.25	274^{cdefgh}	65.7^{fghij}	0.50^{tu}	0.32^{i}
C.26	327^{bcdef}	91.7^{cdefgh}	0.52^{tu}	0.47^{i}
C.27	344^{bcde}	86.0^{cdefghi}	0.42^{uv}	0.37^{i}
C.28	320^{bcdef}	96.0^{bcdefg}	9.77^{h}	9.38^{abc}
C.29	165^{gh}	49.6^{ij}	2.32^{r}	1.12^{i}
C.30	400^{bc}	108^{bcde}	0.46^{uv}	0.49^{i}
C.31	336^{bcdef}	90.7^{cdefgh}	11.0^{d}	10.10^{ab}
C.32	556^{a}	150^{a}	0.35^{v}	0.54^{i}
C.33	387^{bc}	116^{abcd}	4.08^{q}	4.73^{fgh}
C.34	306^{bcdefg}	95.4^{bcdefgh}	12.2^{a}	11.60^{a}
Average	331±80.2	94.2±24.7	5.70±4.47	5.12±4.19
Significant	**	**	**	**
CV (%)	8.63	6.02	3.55	6.79

Values within each column and row followed by the same letter not statistically significant; **1% significance level, C: *Curcuma* and ±: Standard deviation

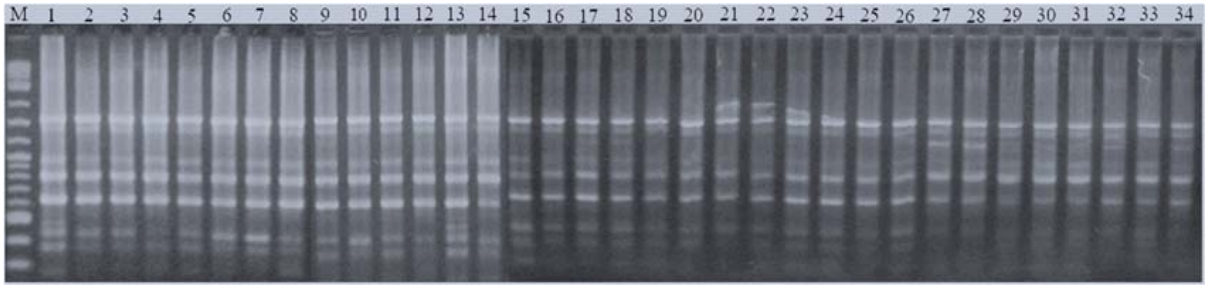

Fig. 4: Result of gel electrophoresis of PCR products obtained by using OPA02 RAPD primer
M: NEB 2 log ladder and well 1-34 as varieties C.1 and C.34

Relationship between yield of curcumin and morphological characters: Samples with low content of curcumin (0.60±0.22) had reddish brown midrib, white or pale yellow flesh or dark purple inner and light purple outer, camphor aroma, bitter taste, dark purple bract or green inferior and reddish purple. According to Lim[23], these samples might belong to *C. aeruginosa* and *C. zedoaria*. Samples with an average content of curcumin (5.14±1.33) had green midrib, yellow or light orange yellow, turmeric aroma, quite bitter and less methylated. The color of the superior bract was light green/ light pink/white. According to Lim[23], these samples might belong to *C. aromatica* and *C. mangga*. Samples with high content of curcumin (10.0±1.09) had green midrib, orange yellow or dark orange yellow flesh, turmeric aroma, quite bitter and less methylated. The color of superior bract was light green, light pink, or white. According to Lim[23] these samples might belong to *C. longa* and *C. xanthorrhiza*.

According to Miyazaki *et al.*[22], the curcumin yield of samples with high curcumin content depended on the weight of dry rhizome while the curcumin yield of samples with low content depended on the curcumin content. The curcumin yield of samples with average curcumin content depended on both dry rhizome weight and curcumin content. Samples with low mass and high content of curcumin had orange flesh while samples with high mass and low content of curcumin had light yellow flesh. Therefore, genotypes affected the content of curcumin and the yield of rhizome and curcumin.

Beyene *et al.*[24] suggested that analyzing diversity based on morphological characters was simple and easy to conduct but it was inaccurate, less effective and could be used for preliminary analysis. In addition, these characteristics are strongly affected by genotype and environment. In this study, the combination of morphological characteristics with biochemical indicators could be used to identify or distinguish different varieties/species. Therefore, to more effectively assess the variety of varieties/species belonging to the genus of the topic, RAPD and ISSR were used.

Molecular markers

RAPD markers: Ten RAPD primers were used to analyze 34 samples of local and imported turmeric, showing that all b ands were polymorphic. As 167 bands were recorded with a mean of 16.7±2.75 band/primer; in which, 155 bands were polymorphic (a ratio of 90.7±18.5%). The number of polymorphic bands ranged from 04 bands (OPA02) to 19 bands (OPD02) with a mean of 15.5±4.28 polymorphic bands per primer (Fig. 4). Most primers produced many bands and had high ratio of polymorphic bands except OPA02 primer (only 4 bands and ratio of polymorphic band was 40.0%).

The PIC index ranged from 0.31 (OPA02) to 0.93 (OPB10) with a mean of 0.59±0.16. The MI index ranged from 6.78 (OPB10) to 12.3 (OPD02) with a mean of 10.4±2.08. The Rp index ranges from 1.18 (OPA02) to 12.8 (OPD02) with a mean of 8.8±3.33 (Table 2).

ISSR markers: Ten ISSR primers were used to analyze 34 samples, showed that all bands were polymorphic. A total of 166 bands were recorded with a mean of 16.6±3.31 bands/primer. In which, 162 bands were polymorphic (with a ratio of 97.1±3.87%). Several polymorphic bands ranged from 9 bands (ISSR1) to 29 bands (ISSR10) with a mean of 16.2±3.61 polymorphic bands per primer. Most primers produced many bands with a high level of polymorphism 90.0% (Table 3). Thus, using 10 ISSR primers to analyze genetic diversity in 34 samples of local and imported turmeric showed the results of higher levels of polymorphism than in the study of Taheri *et al.*[6], Singh *et al.*[25] and Saha *et al.*[26].

Table 2: Index of polymorphic analysis on 10 RAPD primers of 34 samples of local and imported turmeric

Primer	NB	PB	P (%)	PIC	MI	Rp
OPA02	10	4	40.0	0.31	7.00	1.18
OPA03	16	16	100	0.49	11.4	9.29
OPA04	18	18	100	0.55	12.1	10.8
OPA10	17	16	94.1	0.5	10.9	9.90
OPA13	15	14	93.3	0.7	10.9	6.10
OPB07	18	18	100	0.53	11.9	10.9
OPB10	17	17	100	0.93	6.78	6.94
OPD02	19	19	100	0.58	12.3	12.8
OPD03	20	17	85.0	0.73	8.88	9.00
OPD07	17	16	94.1	0.60	11.7	11.0
Total	167	155				
Average	16.7±2.75	15.5±4.28	90.7±18.5	0.59±0.16	10.4±2.08	8.8±3.33

NB: Number of bands, PB: Polymorphic bands, P: Polymorphism, PIC: Polymorphism information content, MI: Marker index, Rp: Resolving power and ±: Standard deviation

Table 3: Index of polymorphic analysis on 10 ISSR primers of 34 samples of local and imported turmeric

Primer	NB	PB	P (%)	PIC	MI	Rp
ISSR1	10	9	90.0	0.50	11.1	5.60
ISSR2	17	17	100	0.67	12.3	11.1
ISSR5	16	15	93.8	0.5	11.1	9.00
ISSR6	20	20	100	0.84	9.9	11.7
ISSR7	14	14	100	0.63	12.4	9.12
ISSR10	22	22	100	0.79	10.8	12.9
ISSR12	16	15	93.8	0.6	11.2	9.70
ISSR14	16	15	93.8	0.7	10.8	9.50
ISSR17	19	19	100	0.69	12.0	11.8
ISSR18	16	16	100	0.68	12.6	11.8
Total	166	162				
Average	16.6±3.31	16.2±3.61	97.1±3.87	0.67±0.10	11.4±0.87	10.2±2.11

NB: Number of bands, PB: Polymorphic bands, P: Polymorphism, PIC: Polymorphism information content, MI: Marker index, Rp: Resolving power and ±: Standard deviation

Table 4: Comparison of RAPD, ISSR and cumulative band data analyses in 34 samples of local and imported turmeric

Marker	RAPD	ISSR	*Cumulative
Total number of primer	10	10	20
Total number of bands amplified	167	166	333
Number of bands/primer	16.7±2.75	16.6±3.31	15.9±2.97
Polymorphic bands	155	162	317
Polymorphic bands/primer	15.5±4.28	16.2±3.61	15.9±3.87
Polymorphism (%)	90.7	97.1	93.9

*Cumulative: Combined data of RAPD and ISSR and ±: Standard deviation

Combination of RAPD and ISSR markers: The results in Table 4 showed that the combination of 10 RAPD primers and 10 ISSR primers in 34 samples of local and imported turmeric had a total of 333 bands with 272 polymorphic bands (93.2%). The subgroup of 34 samples of local and imported turmeric by morphological characters in comparison with RAPD showed similar results (23/34 were identical), with ISSR (17/34 were same) and with the combination of RAPD+ISSR (18/34 were same). In which, C.27 belonged to 1 separate group.

Curcuma aromatica had grey yellow; however, other authors reported that *C. aromatica* had the color of yellow to dark yellow[17]. The morphological differences were caused by the interaction between environment and genotype which were mainly by quantitative factors and rarely by quality characteristics. In addition, turmeric varieties, wrong variety identification and cultivation techniques also strongly affect turmeric morphology. The results showed that the use of RAPD and ISSR molecular markers is very effective and reliable in assessing genetic diversity, relationships and classification of 34 local and imported turmeric varieties. In summary, using morphological characteristics, agronomic traits and molecular markers the genetic relationship of the C.34 turmeric sample (belonging to *Curcuma xanthorrhiza* species) was identified.

CONCLUSION

Thirty fours samples of local and imported turmeric were split into three groups (group I: *C. zedoaria*; group II:

C. aeruginosa; group III: *C. xanthorrhiza*, *C. longa* and *C. mangga*. The research has selected the C.34 sample with the highest curcumin content and yield per dry rhizome (12.2% and 11.6 g, respectively). The C.34 sample has leaf midrib; orange/orange-yellow rhizome colour, aroma of turmeric and little bitter taste of rhizome; the colour of the coma bract is green/light pink/white, classified as *Curcuma xanthorrhiza* species. In the future, the C.34 variety could be cultivated for curcumin's purpose.

SIGNIFICANCE STATEMENT

The study was carried out to identify varieties of with high yield and curcumin content based on its morphology, molecular and biochemical markers. In Vietnam, turmeric is a spice crop that has several uses and is commercially significant. There are several local and imported varieties of turmeric planted with curcumin's purpose. Based on morphological characteristics, it is possible to estimate the content and yield of curcumin/weight of dry rhizome of 34 local and imported turmeric varieties. *Curcumin xanthorrhiza* species (C.34 variety) with the highest curcumin content and yield on dry rhizomes (12.4%, 11.6 g).

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