

Short Communication: Comparisons of isozyme diversity in local Java cardamom (*Amomum compactum*) and true cardamom (*Elettaria cardamomum*)

AHMAD DWI SETYAWAN^{1,*}, WIRYANTO¹, SURANTO¹, NURLIANI BERMAWIE², SUDARMONO³

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Jl. Ir. Sutami 36a, Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-663375, *email: volatileoils@gmail.com

²Indonesian Medicinal and Aromatic Crops Research Institute, Jl. Tentara Pelajar No.3, Cimanggu, Bogor 16111, West Java, Indonesia.

³Bogor Botanic Garden, Indonesian Institute of Sciences, Jl. Ir. H. Juanda No.13, Bogor 16122, West Java, Indonesia.

Manuscript received: 13 February 2014. Revision accepted: 28 April 2014.

Abstract. Setyawan AD, Wiryanto, Suranto, Bermawie N, Sudarmono. 2014. Comparisons of isozyme diversity in local Java cardamom (*Amomum compactum*) and true cardamom (*Elettaria cardamomum*). *Nusantara Bioscience* 6: 94-101. Fruits of Java cardamoms (*Amomum compactum*) and true cardamoms (*Elettaria cardamomum*) had long been used as spices, flavoring agent, garnishing plants etc. This research was conducted to find out: (i) variation of isozymic bands in some population of Java cardamoms and true cardamoms; and (ii) phylogenetic relationship of these cardamoms based on variation of isozymic bands. Plant material (i.e. rhizome) of Java cardamoms was collected from Bogor Botanical Garden, and plant material of true cardamoms was gathered from Indonesian Medicinal and Aromatic Crops Research Institute, Bogor, Indonesia. Ten accessions were assayed in every population. The two isozymic systems were assayed, namely esterase (EST) and peroxidase (PER, PRX). Phylogenetic relationship was determined by UPGMA method. The results showed that esterase gave nine isozymic bands, i.e. Rf 0.15, 0.26, 0.29, 0.33, 0.38, 0.42, 0.45, 0.53, and Rf 0.58., while peroxidase gave 10 isozymic bands, i.e. Rf 0.06, 0.14, 0.18, 0.22, 0.26, 0.29, 0.32, 0.37, 0.41, and Rf 0.46. Relationship dendrogram indicated that the number of data will affect the grouping based on species similarity; and more data was increasingly apparent in groupings; within these groups there were variations among its members.

Key words: *Amomum compactum*, cardamoms, *Elettaria cardamomum*, isozyme, Zingiberaceae

INTRODUCTION

Cardamom is one of the most expensive and most pleasantly scented spices in the world (after saffron and vanilla). Cardamom is used as spices, condiments, perfumes, cosmetics, traditional medicine, pharmaceutical, food and beverage (Heyne 1950). Cardamom produced by three genera of Zingiberaceae, namely *Amomum* (four of 176 sp.), *Aframomum* (three of 54 sp.), and *Elettaria* (one of 11 sp.). World markets provide three types of cardamoms, i.e. green, black and Madagascar cardamoms. Green cardamom (or true cardamom) is produced by *Elettaria cardamomum* var. *cardamomum*, which is native to southern Asia. It is the most sought after of all the species and the most expensive, where 80% of the world market fulfilled. Green cardamom of Sri Lanka (Ceylon cardamom) is produced by less quality of *E. cardamomum* var. *major*. Black cardamom is produced by *Amomum*, which has distribution from India, China and Southeast Asia to Australia, includes *Amomum aromaticum* (Bengali cardamom), *A. compactum* (Java, round, or Siam cardamom), *A. subulatum* (Greater Indian or Nepal cardamom) and *A. testaceum* (Cambodian cardamom). Madagascar or Cameroon cardamom is produced by *Aframomum angustifolium*, which is the most distributed cardamoms of tropical Africa. *Aframomum corrarima* (Korarrima cardamom) and *A. melegueta* (grains of paradise or Guinea grains) are other cardamoms of

Africa (Heyne 1950; Backer and Bakhuizen v.d. Brink 1968; Purseglove 1972; Purseglove et al. 1981; Wolff and Hartutiningsih 1999; Wardini and Thomas 1999; Peter 2001; Duke et al. 2002).

In Indonesia, there are two types of cardamom, i.e. local Java cardamom (*Amomum compactum* Soland ex. Maton.) and true cardamom (*Elettaria cardamomum* (L.) Maton.; syn. *Amomum cardamomum* L.). Java cardamom is an endemic species of West Java and is now cultivated throughout Southeast Asia and South China. Meanwhile, true cardamom comes from Malabar Mountains of western India and introduced to Indonesia since the 1920s, and is cultivated commercially in 1986 (Heyne 1950; Seidemann 2005). Most Indonesian farmers cultivate Java cardamom. True cardamom is less successfully cultivated because of differences in microclimate and soil factors, except in Tasikmalaya of West Java, Kulonprogo of Yogyakarta and West Sumatra (Santoso 1988; Madjo-Indo 1989). True cardamom has better aromatic quality because of higher content of volatile oil (5-8%), and Java cardamom has less oil (2-3.5%) (Santoso 1988). Other literatures stated that volatile oil of true cardamom are 3.5-7% (Guenther 1952), 2-8% (Hegnauer 1963; Purseglove 1972), 3-7% (Youngken 1948), or 2.8-6.2% (Trease and Evans 1978). The results were confirmed by Setyawan (2000), where essential oil content of true cardamom is higher (2.25%) than the Java cardamom (1.5%). Genetic,

environmental condition, method of oil extraction influenced oil yield.



Figure 1. The morphology of *A. compactum*: A. Clumps, B. Flower, C. Fruits, D. Seeds (pods); and *E. cardamomum*: E. Clumps, F. Flower, G. Fruits, H. Seeds (pods). (photos from many sources)

The major components of true cardamom seeds are α -terpinyl acetate (~40%), 1,8-cineole (20-25%), and linalool (5-6%) (Marongiu et al. 2004; Kuyumcusavan and Kucukbay2013). The other components include borneol, linalyl acetate, limonene, linalool, α -terpinen, terpinolene, and myrcene (Marongiu et al. 2004). While, the major components of volatile oils of Java cardamom is cineole (60-80%). The other components include α -pinene, β -pinene, camphene, limonene, ρ -cymene, α -terpineol and α -humulene (Yu et al. 1982; Feng et al. 2011). True cardamom is contains more α -terpinyl acetate than Java cardamom, a valuable fragrant liquid ester.

True cardamom and Java cardamom can be distinguished easily by morphological characteristics. True cardamom plant is higher (1.5-4 m) than Java cardamom (1-2.5 m). The base of true cardamom stem is light green, while Java cardamom is reddish green. True cardamom leaves are lancet-oblong (tapered leaf tip and base); while Java cardamom leaves are lancet. Inflorescence of true cardamom is raceme or botrys, while the Java cardamom is capitulum. Flower stalk of true cardamom has a length of 50-120 cm; spread on the soil surface (Mysore cultivar) or upright (Malabar cultivar). Flower stalk of Java cardamom is very short, as if no stalk at all. The true cardamom pods are green, while the Java cardamom pods are reddish-white and became brownish-black when dry. True cardamom

pods are oblong-triangular, while the Java cardamom pods are round and slightly flattened (Santoso 1988; Madjo-Indo 1989)(Figure 1).

Research of cardamom variability using isozymic pattern has not been done yet, except for *Amomum aromaticum*, *A. cannicarpum*, *A. kingii*, and *A. subulatum* (Shanmugapriya and Prabha2012), while the same research on other ginger family had been conducted to *Boesenbergia* (Vanijajiva et al. 2003), *Curcuma alismatifolia* (Paisooksantivatana 2001), *C. aeruginosa*, *C. elata*, *C. rubescens*, *C. zedoaria* (Apavatjirut et al. 1999), *C. chuanhuangjiang*, *C. kwangsiensis*, *C. phaeocaulis*, *C. wenyujin* (Tang et al. 2008; Deng et al. 2011), *C. sichuanensis* (Deng et al. 2011), *C. longa* (Shamina et al. 1998; Deng et al. 2011), *C. xanthorrhiza* (Apavatjirut et al. 1999; Azizah 2011), *Hedychium spicatum* (Jugran et al. 2011), *Zingiber officinale* (Shamita1997; Setyawan et al. 2014 in this issue), and a few other species of *Curcuma* (Chokthaweeapanichand Paisooksantivatana2003) and other species of Zingiberaceae (Ibrahim et al. 1991; 1996).

The aim of this study is to determine (i) the isozymic diversity of local Java cardamom (*Amomum compactum* Soland ex. Maton.) and true cardamom (*Elettaria cardamomum* (L.) Maton.) based on isozymic pattern of esterase and peroxidase, and (ii) the genetic

relationship between two species based on the isozymic patterns.

Materials and methods

Plants material. Plant material (i.e. rhizome) of Java cardamoms (*Amomum compactum* Soland ex. Maton.) was collected from Bogor Botanical Garden, and plant material of true cardamoms (*Elettaria cardamomum*) was gathered from Indonesian Medicinal and Aromatic Crops Research Institute (IMACRI/Balittro) Bogor, West Java, Indonesia. From each species, 10 accessions were assayed. A series of observations was also conducted on 10 accessions of Java cardamom from the Medicinal Plant and Traditional Medicine Research and Development Center (B2P2TOOT) Tawangmangu, Central Java, Indonesia, but they showed no variation (especially with peroxidase), thus it is ignored in the data analysis. They are probably the sibling of the same accession which is separated vegetatively (Figure 2.E-F).

The rhizome was harvested from the two years old mature crops and then planted. The plant materials were authenticated at the Department of Biology, Sebelas Maret University, Surakarta, Indonesia. Enzyme staining systems were esterase (EST) and peroxidase (PER, PRX), and separated on a polyacrylamide gel.

Procedures. The electrophoresis procedure refers to Crawford (1990) and Weeden and Wendel (1989) as modified by Suranto (1991).

Planting of rhizomes. The rhizome was placed on a plastic tray lined with wet paper or cloth to keep moisture, until the leaves grow and reach 2-5 mm. Shoots were cut and immediately used for examination, or were stored in a refrigerator at 4°C for a maximum of 14 days, but can only be used effectively within seven days after cutting. Leaf extracts that stored in a refrigerator at 4°C can survive for 30 days.

Buffer. Tank buffer was made by dissolving 14.4 g of boric acid and 31.5 g of borax (sodium borate), in distilled water to a volume of 2 L. Extraction buffer was made by dissolving 0.018 g of cysteine, 0.021 g of ascorbic acid, and 5 g of sucrose (PA) in 20 mL of borax buffer at pH 8.4. Running buffer was TAE (Tris-Acetic Acid-EDTA)

50x diluted to a concentration of 1x.

Preparation of gel. *First stock solution:* 27.2 g Tris and 0.6 g SDS dissolved in 120 mL of distilled water; it is adjusted to pH 8.8 by adding HCl, then is added with distilled water up to 150 mL. *Second stock solution:* 9.08 g Tris and 0.6 g SDS dissolved in 140 mL of distilled water, adjusted to pH 6.8 to 7.0 by adding HCl, then added distilled water up to 150 mL. *Third stock solution:* 175.2 g of acrylamide and 4.8 g bis-acrylamide are dissolved in 400 mL of distilled water and then make up to 600 mL. *Loading dye:* 250 μ L of glycerol and 50 μ L bromophenol blue (BPB) dissolved in 200 μ L of distilled water. *Separating gel:* 3.15 mL of the first stock solution and 5.25 mL of the second stock solution, added with 4.15 mL of distilled water, 5 μ L of TEMED, and 10 μ L of APS 10% (new). The mixed solution was poured into the mold, then added with saturated isobutanol. When the gel was formed (~45 minutes), saturated isobutanol was absorbed by blotting paper. *Stacking gel:* 1.9 mL of the second stock solution and 1.15 mL of the third stock solution, added with 4.5 mL of distilled water, 5 μ L TEMED, and 10 μ L APS 10% (new). Stacking gel was poured above the separation gel, fitted with a comb to make wells, that was released after gel formation. The formed gel was transferred into the clamping frame and put in a buffer tank, then filled with running buffer until submerged.

Extraction. Fresh leaf tissue was put in the extraction buffer, with a ratio of 1:4 (w/v), i.e. 68 μ g (0.068 g) of leaf samples were pulverized in 272 μ L (0.272 mL) of extraction buffer. Then it is crushed in a porcelain dish that was placed above ice crystals, to keep it cold (4°C). Samples were centrifuged at 8500 rpm for 20 minutes at 4°C, then soaked in ice crystals. Supernatant was put in the well of gel.

Electrophoresis. 3.5 μ L supernatant was added with loading dye and sample loading guide, and then placed in the wells. Samples were electrophoresed at 200 volts, 60 mA for 5 minutes to reach the separating gel, and electrophoresed at 150 V, 400 mA, for 60 minutes, i.e. loading dye reaches ~56 mm from the well toward anode. Gel was transferred into a plastic tray and colored with enzyme dyes.

Staining. *Peroxidase:* 0.0125 g of O-Dianisidine was put into Erlenmeyer and dissolved with 2.5 mL of acetone, and then was added with 50 mL of 0.2 M acetate buffer pH 4.5 and 2 drops of H₂O₂. *Esterase:* 0.0125 g of α -Naphthyl acetate was put into Erlenmeyer and dissolved with 2.5 mL of acetone, and then was added with 50 mL of 0.2 M phosphate buffer pH 6.5 and 0.0125 g Fast Blue BBS salt. Separately, gel was soaked to those solutions for 10 minutes and was shaken gently every 2 minutes.

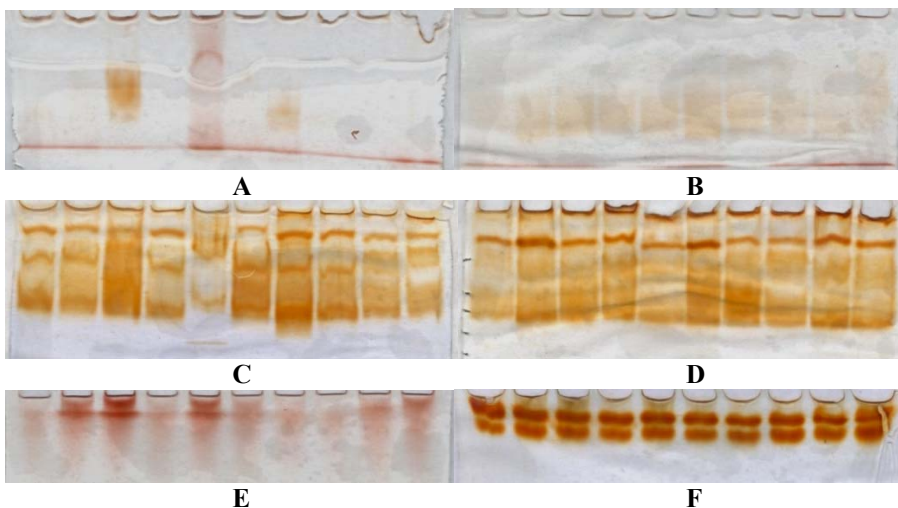


Figure 2. Zimogram of cardamoms. Java cardamom (A) and true cardamom (B) stained with esterase. Java cardamom (C) and true cardamom (D) stained with esterase. Java cardamom of B2P2TOOT Tawangmangu stained with esterase (E) and peroxidase (F).

Once the banding pattern appeared, the enzyme was disposed and rinsed with distilled water, then it is recorded by a camera or scanner.

Data analysis. Only clear, unambiguous and reproducible bandings were considered for data analysis. Rf (*retardation factor*) value was calculated based on the relative movement of isozyme and loading dye. Present bands were given a value of 1, while those absences were given a value of 0. Data were entered in a spreadsheet to create a binary matrix. The genetic similarity among the accessions was calculated according to Jaccard (1908) coefficients. The matrices were computed and corresponding dendrograms of genetic relatedness were constructed by applying un-weighted pair group method with arithmetic mean (UPGMA) clustering algorithm (Sneath and Sokal 1973), using the Dendro-UPGMA program (Garcia-Vallvé and Puigbo 1999; Garcia-Vallve et al. 2002).

Results and discussion

Indonesia is home to at least 20 members of the genus of *Amomum*, where one of them, *Amomum compactum*, has a high economic value and is commonly known as Java cardamom (Kasahara and Hemmi 1995). In international trade, the local cardamom got strong competition from true cardamom, *Elettaria cardamomum*, which originated and widely cultivated in India and Sri Lanka. True cardamom tends to be less suited to the microclimate and soil type in Indonesia. Therefore, it is necessary for genetic improvement of native Indonesian cardamom to compete with true cardamom which now dominates world trade. Identification and characterization of the diversity of cardamom is required to improve the quality and quantity of production.

Variation of isozymic pattern

Esterase (EST). Esterase isozyme indicates the presence of nine isozymic banding located at Rf 0.15, 0.26, 0.29, 0.33, 0.38, 0.42, 0.45, 0.53, and 0.58 (Figure 2-3). Bands are from pink to brown. The bands are generally very thin, thus it should be repeated 2-3 times to ensure consistency. This is in contrast with observations on the esterase isozyme of ginger, which bands are from purple-blue to red, and generally quite thick that they are easily observed (Setyawan et al. 2014). On the other hand, observations of esterase isozymic bands on the sample of Java cardamom taken from the collection of B2P2TOOT Tawangmangu just generate 2-3 clear bands from the total of 10 accessions. No variation between accessions is thought to occur because they are derived from vegetative propagation from the same parent. Vegetative propagation generally results in low diversity (Jia and Sun 2013). Therefore, this material is not used for further data analysis.

In this research, the most frequent appeared bands are located at Rf 0.53, this band was not detected in one Java cardamom accession, and two true cardamom accessions. Another band appears quite often lies in Rf 0.45. This band appears on all accessions of true cardamom, but only appeared in one Java cardamom accession. The most distinctive band because it only appears on one accession and quite thick is located at Rf 0.15. The bands belong to accession no. 11 of Java cardamom. The band distribution pattern of this accession is very specific that makes it one of the most distinctive accessions because its genetic diversity is relatively different from the other accessions. In this research, it is also found accessions having identical isozymic banding pattern, namely: two accessions of Java cardamom (5, 9) and three accessions of true cardamom (16, 17, 18). However, no two accessions of different species have identical band.

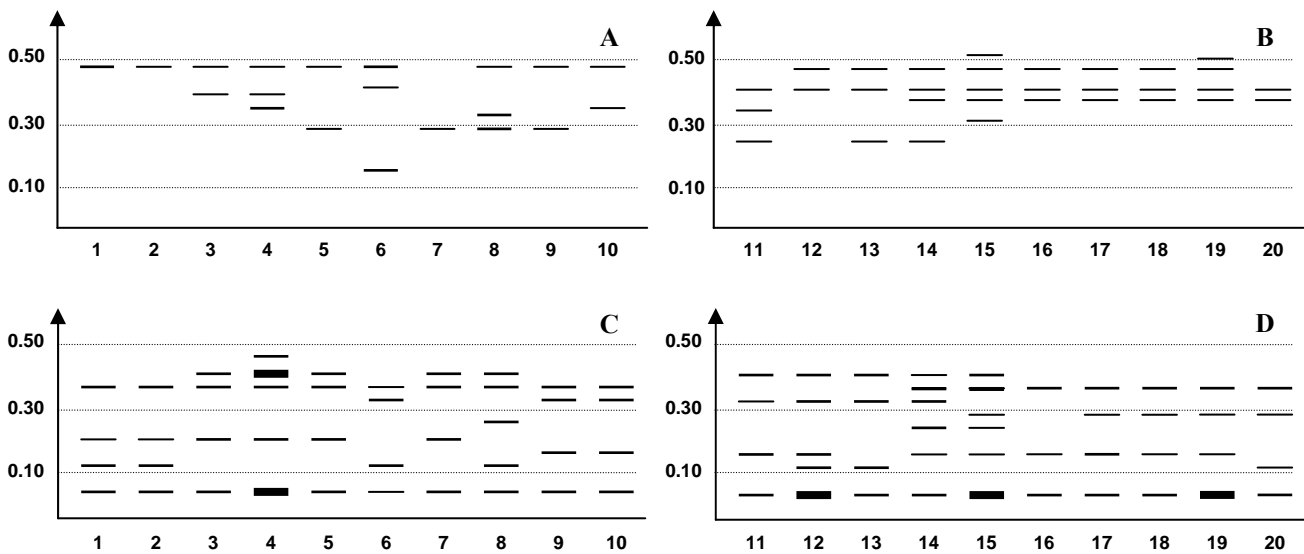


Figure 3. Schematic zimogram of cardamoms. Esterase isozymic pattern of Java cardamom (A) and true cardamom (B). Peroxidase isozymic pattern of Java cardamom (C) and true cardamom (D).

The number of esterase bands that emerged in this research are quite many (nine bands) compared to some other studies. Esterase in higher plants usually gives only 3-4 isozymic bands. Esterase in some *Amomum* species raises only one isozymic band, and unable to demonstrate the existence of variation between species (Shanmugapriya and Prabha 2012). Two isozymic bands were raised by application of esterase in three varieties of ginger, i.e. small white, large white and small red gingers (Setyawan et al. 2014). Application of esterase in several species of *Curcuma* are also only produces 1-4 isozymic bands depend on the species (Apavatjirut et al. 1999).

Peroxidase (PER, PRX). Peroxidase leads to quite a lot of variation in isozymic bandings. Observation on peroxidase isozyme shows 10 bands, namely at Rf 0.06, 0.14, 0.18, 0.22, 0.26, 0.29, 0.32, 0.37, 0.41, and 0.46 (Figure 2-3). It forms reddish brown and relatively thick bandings so that it is easily observed. However, from the collection of all accessions of Java cardamom from B2P2TOOT Tawangmangu, only two peroxidase bands appear with the same Rf, which indicate monomorphic (Figure 2.F). The application is repeated 2-3 times on these accessions but the results were consistently the same, so it is suspected that they derived from the same and homozygote mother plant. Peroxidase isozymic band obtained from samples collected from Tawangmangu is much thicker than the peroxidase isozymic bands obtained from samples from Java cardamom of IMACRI Bogor or true cardamom from the Bogor Botanical Gardens. The environmental condition where the plants were grown may influence the peroxidase activity. Higher activity was found from samples collected from higher elevation. Tawangmangu located > 1000 m above sea level, while IMACRI and Botanical Garden were both in Bogor with elevation 250 m above sea level.

In this research, each accession gives a fair amount of peroxidase isozymic bands, ranging from 3-5 bands per accession. Isozymic banding that appears in all of the accession lies in Rf 0.06, but the quality varies from one accession to another, namely: thin, medium and thick, thus the banding can still be used as distinguishing character in the preparation dendrogram. Other isozymic banding with a fairly high frequency lies in Rf 0.37. The band is present in all accessions of Java cardamom, and seven accessions of true cardamom. Isozymic banding with the lowest frequency is at Rf 0.22, which only presents on one accession of Java cardamom and two accessions of true cardamom. A fairly typical banding pattern is found in accession 11 of Java cardamom and the accession 16 of true cardamom; whose band pattern is different from other accessions. Meanwhile, some accessions have identical bands, i.e. three groups of accessions of Java cardamom (1, 2), (9, 10) and (3, 5, 7), as well as a group of accessions of true cardamom (16, 18). Similarly with the esterase bands, no two accessions of different species have identical peroxidase bands.

In this research, the amount of peroxidase bands detected is 10 isozymic bands. In higher plants, the enzyme is usually only generate 4-6 bands. Research on *Amomum* suggests that this enzyme only gave 4 isozymic bandings

(Shanmugapriya and Prabha 2012). While, in the research on different varieties of ginger which are distinguished by the color of rhizome, these enzymes can bring 6 isozymic bandings (Setyawan et al. 2014). It was known that peroxidase always produce more isozymic bands than esterase do. This suggests that the reactions catalyzed by the peroxidase enzyme are more diverse than the reactions catalyzed by the esterase enzyme.

Phylogenetic relationship

Esterase (EST). Esterase enzyme gives a lot isozymic bands i.e. nine bandings which is located at Rf 0.15, 0.26, 0.29, 0.33, 0.38, 0.42, 0.45, 0.53, and 0.58. That large number of isozymic bands causes variety in the formation of dendrogram relationship. However, there are also accessions with identical band, i.e. the four accessions of Java cardamom in two groups, i.e. (1, 2) and (5, 9) and three accessions of true cardamom (16, 17, 18), thus that the dendrogram indicates the absence of distance (Figure 4.A; Table 1.A). In this research, the dendrogram has a cascade form, it indicates that the degree of separation by esterase enzymes are not good enough, thus the grouping of accessions based on similarities between species are not quite shown. Nonetheless, some accessions have been grouped by species, for example, five accessions of Java cardamom (1, 2, 3, 4, 10) and four accessions of Java cardamom (5, 7, 8, 9). Besides, almost all accessions of true cardamom clustered in one group. Within this group, six accessions of true cardamom (15, 16, 17, 18, 19, 20) generate a sub-group, while in the other sub-group, its members still mixed between the two species, i.e. three accessions of true cardamom (12, 13, 14) and one accession of Java cardamom (6). Meanwhile, one accession of true cardamom emerge it self (11). Based on zimogram, accession 11 has a very distinctive esterase banding pattern (Figure 4.A).

Peroxidase (PRX, PER). Peroxidase enzyme brings up 10 isozymic banding i.e. at Rf 0.06, 0.14, 0.18, 0.22, 0.26, 0.29, 0.32, 0.37, 0.41, and Rf 0.46. The amount of isozymic bands leads to inter-accession variability which is reflected properly. Groups of accessions generally gather with the basis of species similarity. There is two group whose members are from the same species, which is Java cardamom (3, 4, 5, 7) and true cardamom (11, 12, 13). However, in general there are other groups of one to two members of different species; for example, a group of Java cardamom (1, 2, 6, 8) are still mixed with an accession of true cardamom (20). Conversely, there is also a group of true cardamom (14, 15, 16, 17, 18, 19) mixed with two accessions of Java cardamom (9, 10) (Figure 4.B; Table 1.B). In general, the peroxidase enzyme reveals more isozymic banding than esterase enzyme does, thus it gives a better overview of the diversity between accessions and the tendency of clustering among accessions based on species similarity. Esterase enzyme gives lesser amount of isozymic banding than the peroxidase enzyme does, thus dendrogram made by esterase isozymic banding reflects less the grouping based on similarity of species compared to dendrogram made by peroxidase isozymic banding.

Combination of esterase and peroxidase. Relationship dendrogram formed by the combined characteristics of esterase and peroxidase isozymes utilizes more data genetic diversity, thus dendrogram formed reflects more the grouping based on similarity of species. However, the dendrogram also shows variation between accessions of the same species (Figure 4.C). The combined characters formed three groups. One group is consisted of only Java cardamom (1, 2, 3, 4, 5, 7, 8), one group is only true

cardamom (14, 15, 16, 17, 18, 19, 20), and a third group consisting of either Java or true cardamoms. The latter group also reflects the presence of another grouping based on similarity of species, namely: a group of Java cardamom (9, 10), and a mixed group consisting of true cardamom (11, 12, 13) and the Java cardamom (6). Meanwhile, accession11 of true cardamom is the most different accession in this last group (Table 2).

Table 1. Matrixs of similarity computed with Jaccard coefficient on species of Java cardamom and true cardamom based on isozymic pattern of esterase and peroxidase.

A. Similarity matrix of esterasebanding pattern

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	1.000	0.600	0.500	0.600	0.600	0.600	0.500	0.333	0.333	0.143	0.286	0.333	0.250	0.286	0.400	0.400	0.400	0.400	0.750
2	1.000	1	0.600	0.500	0.600	0.600	0.600	0.500	0.333	0.333	0.143	0.286	0.333	0.250	0.286	0.400	0.400	0.400	0.400	0.750
3	0.571	0.571	1	0.800	1.000	0.333	1.000	0.500	0.333	0.333	0.333	0.286	0.333	0.429	0.500	0.400	0.400	0.400	0.400	0.400
4	0.444	0.444	0.750	1	0.800	0.286	0.800	0.429	0.286	0.286	0.286	0.250	0.286	0.375	0.429	0.333	0.333	0.333	0.333	0.333
5	0.571	0.571	0.714	0.556	1	0.333	1.000	0.500	0.333	0.333	0.333	0.286	0.333	0.429	0.500	0.400	0.400	0.400	0.400	0.400
6	0.500	0.500	0.300	0.250	0.300	1	0.333	0.500	0.600	0.600	0.333	0.500	0.600	0.429	0.286	0.400	0.400	0.400	0.400	0.750
7	0.429	0.429	0.571	0.444	0.833	0.200	1	0.500	0.333	0.333	0.333	0.286	0.333	0.429	0.500	0.400	0.400	0.400	0.400	0.400
8	0.444	0.444	0.400	0.333	0.556	0.364	0.444	1	0.286	0.286	0.286	0.429	0.500	0.375	0.429	0.333	0.333	0.333	0.333	0.600
9	0.375	0.375	0.333	0.273	0.500	0.444	0.375	0.400	1	1.000	0.600	0.500	0.333	0.667	0.500	0.750	0.750	0.750	0.750	0.400
10	0.375	0.375	0.333	0.400	0.333	0.444	0.222	0.273	0.714	1	0.600	0.500	0.333	0.667	0.500	0.750	0.750	0.750	0.750	0.400
11	0.091	0.091	0.182	0.250	0.182	0.273	0.200	0.154	0.300	0.444	1	0.800	0.600	0.667	0.500	0.400	0.400	0.400	0.400	0.167
12	0.333	0.333	0.300	0.250	0.300	0.556	0.200	0.364	0.444	0.444	0.556	1	0.800	0.571	0.429	0.333	0.333	0.333	0.333	0.333
13	0.333	0.333	0.300	0.250	0.300	0.556	0.200	0.364	0.300	0.300	0.556	0.750	1	0.429	0.286	0.167	0.167	0.167	0.167	0.400
14	0.250	0.250	0.455	0.385	0.333	0.417	0.250	0.286	0.455	0.455	0.545	0.545	0.545	1	0.833	0.500	0.500	0.500	0.500	0.286
15	0.250	0.250	0.455	0.385	0.333	0.308	0.250	0.385	0.333	0.333	0.308	0.417	0.308	0.667	1	0.600	0.600	0.600	0.600	0.333
16	0.375	0.375	0.500	0.400	0.333	0.444	0.222	0.273	0.500	0.500	0.300	0.444	0.300	0.600	0.600	1	1.000	1.000	1.000	0.500
17	0.375	0.375	0.500	0.400	0.333	0.444	0.222	0.273	0.500	0.500	0.300	0.444	0.300	0.600	0.600	1.000	1	1.000	1.000	0.500
18	0.375	0.375	0.500	0.400	0.333	0.444	0.222	0.273	0.500	0.500	0.300	0.444	0.300	0.600	0.600	1.000	1.000	1	1.000	0.500
19	0.333	0.333	0.444	0.364	0.300	0.400	0.200	0.250	0.444	0.444	0.273	0.400	0.273	0.545	0.700	0.857	0.857	0.857	1	0.500
20	0.429	0.429	0.375	0.300	0.222	0.500	0.250	0.300	0.222	0.222	0.200	0.333	0.333	0.364	0.364	0.571	0.571	0.571	0.500	1

B. Similarity matrix of peroxidasebanding pattern

Note: 1-10. Java cardamom, 11-20. True cardamom.

Table 2. Matrixs of similarity and distance computed with Jaccard coefficient on species of Java cardamom and true cardamom based on combination of isozymic pattern of esterase and peroxidase.

Similarity matrix of esteraseand peroxidase banding patterns

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1		1.000	0.500	0.333	0.500	0.333	0.000	0.333	0.500	0.500	0.000	0.500	0.333	0.250	0.200	0.333	0.333	0.333	0.250	0.000
2	0.000		0.500	0.333	0.500	0.333	0.000	0.333	0.500	0.500	0.000	0.500	0.333	0.250	0.200	0.333	0.333	0.333	0.250	0.000
3	0.500	0.500		0.667	0.333	0.250	0.000	0.250	0.333	0.333	0.000	0.333	0.250	0.500	0.400	0.667	0.667	0.667	0.500	0.333
4	0.667	0.667	0.333		0.250	0.200	0.000	0.200	0.250	0.667	0.200	0.250	0.200	0.400	0.333	0.500	0.500	0.500	0.400	0.250
5	0.500	0.500	0.667	0.750		0.250	0.500	0.667	1.000	0.333	0.000	0.333	0.250	0.200	0.167	0.250	0.250	0.250	0.200	0.000
6	0.667	0.667	0.750	0.800	0.750		0.000	0.200	0.250	0.250	0.200	0.667	0.500	0.400	0.333	0.500	0.500	0.500	0.400	0.250
7	1.000	1.000	1.000	1.000	0.500	1.000		0.333	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8	0.667	0.667	0.750	0.800	0.333	0.800	0.667		0.667	0.250	0.000	0.250	0.200	0.167	0.333	0.200	0.200	0.200	0.167	0.000
9	0.500	0.500	0.667	0.750	0.000	0.750	0.500	0.333		0.333	0.000	0.333	0.250	0.200	0.167	0.250	0.250	0.250	0.200	0.000
10	0.500	0.500	0.667	0.333	0.667	0.750	1.000	0.750	0.667		0.250	0.333	0.250	0.200	0.167	0.250	0.250	0.250	0.200	0.000
11	1.000	1.000	1.000	0.800	1.000	0.800	1.000	1.000	1.000	0.750		0.250	0.500	0.400	0.143	0.200	0.200	0.200	0.167	0.250
12	0.500	0.500	0.667	0.750	0.667	0.333	1.000	0.750	0.667	0.667	0.750		0.667	0.500	0.400	0.667	0.667	0.667	0.500	0.333
13	0.667	0.667	0.750	0.800	0.750	0.500	1.000	0.800	0.750	0.750	0.500	0.333		0.750	0.333	0.500	0.500	0.500	0.400	0.250
14	0.750	0.750	0.500	0.600	0.800	0.600	1.000	0.833	0.800	0.800	0.600	0.500	0.250		0.500	0.750	0.750	0.750	0.600	0.500
15	0.800	0.800	0.600	0.667	0.833	0.667	1.000	0.667	0.833	0.833	0.857	0.600	0.667	0.500		0.600	0.600	0.600	0.800	0.400
16	0.667	0.667	0.333	0.500	0.750	0.500	1.000	0.800	0.750	0.750	0.800	0.333	0.500	0.250	0.400		1.000	1.000	0.750	0.667
17	0.667	0.667	0.333	0.500	0.750	0.500	1.000	0.800	0.750	0.750	0.800	0.333	0.500	0.250	0.400	0.000		1.000	0.750	0.667
18	0.667	0.667	0.333	0.500	0.750	0.500	1.000	0.800	0.750	0.750	0.800	0.333	0.500	0.250	0.400	0.000	0.000		0.750	0.667
19	0.750	0.750	0.500	0.600	0.800	0.600	1.000	0.833	0.800	0.800	0.833	0.500	0.600	0.400	0.200	0.250	0.250	0.250		0.500
20	1.000	1.000	0.667	0.750	1.000	0.750	1.000	1.000	1.000	1.000	0.750	0.667	0.750	0.500	0.600	0.333	0.333	0.333	0.500	

Distance matrix of esteraseand peroxidase banding patterns

Note: 1-10. Java cardamom, 11-20. True cardamom.

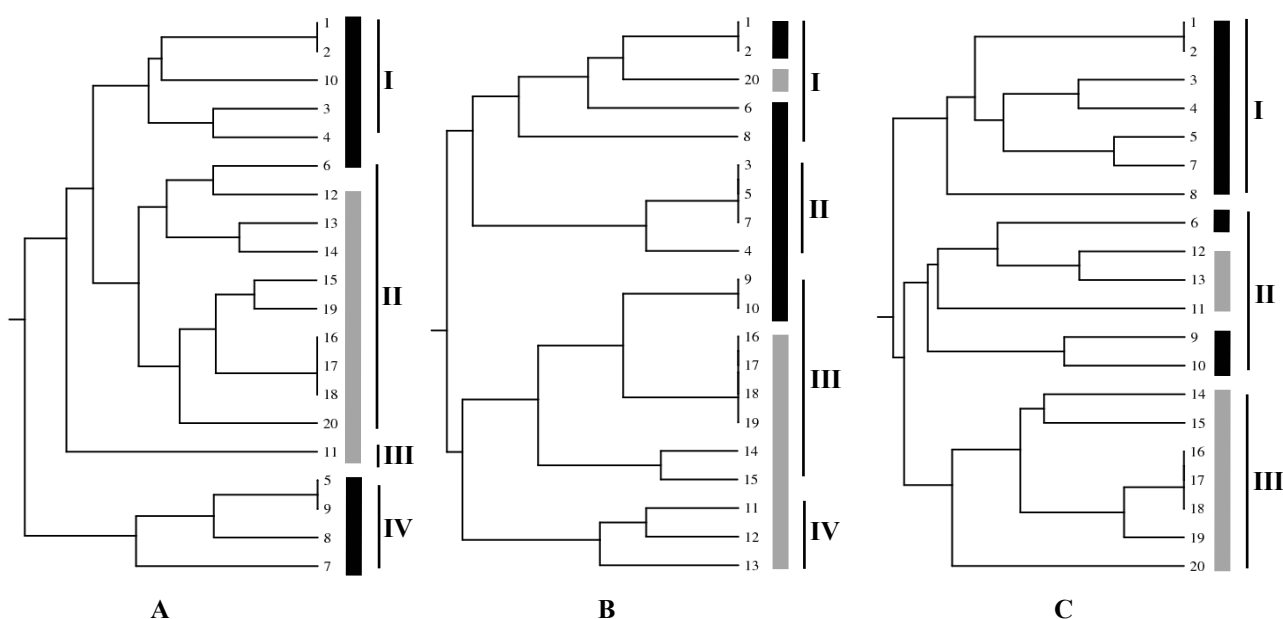


Figure 4. Relationship dendrogram of Java cardamom and true cardamom based on isozymicbanding pattern of esterase (A), peroxidase (B) and combination of esterase and peroxidase (C). Note: ■ = Java cardamom, ■ = true cardamom.

Accession 11 of Java cardamom has a unique relationship position because it tends to stand alone; it is found both in the dendrogram constructed based on esterase isozymic banding, or a combination of both. Generally, accession which has a distinctive genetic pattern is only able to grow in a particular environment, but if that accession is able to grow in a variety of habitat conditions, it must be very valuable because its distinctive genetic variations enable it to withstand changes in the environment. This accession is much needed in plant breeding programs, either in an effort to increase the quality and quantity of production, as well as in the prevention of pests and diseases and global environmental changes such as land use change and global warming.

ACKNOWLEDGEMENTS

This work was supported by the DGHE Republic of Indonesia with research grant no. 031/PUT/DPPM/PDM/III/2003. The authors thank to the Indonesian Medicinal and Aromatic Crops Research Institute (IMACRI/Balitro) Bogor, the Bogor Botanical Garden, and the Medicinal Plant and Traditional Medicine Research and Development Center (B2P2TOOT) Tawangmangu, Indonesia for providing materials of cardamoms. The authors also thank to Suhar Iriyanto and Delima S. Roesyat for his generosity in providing some plant materials, and Asriyati A. Wardani and Ainur Rohimah for kindly assistance with the laboratory work.

REFERENCES

- Apavatjirut P, Anuntalabhochai S, Sirirungsa P, Alisi C. 1999. Molecular markers in the identification of some early flowering *Curcuma* L. (Zingiberaceae) species. *Annual of Botany* 84: 529-534.
- Azizah N. 2011. Identification of genetic diversity on *Curcuma xanthorrhiza* collection clones in Java and Madura. The 2nd International Symposium on Temulawak, IPB International Convention Center 26-27 May 2011
- Backer CA, Bakhuizen van den Brink Jr. RC. 1968. *Flora of Java*. Vol. 3. Wolters Noordhoff, Groningen.
- Chokthaweeapanich H, Paisooksantivatana Y. 2003. Classification of the genus *Curcuma* L. (Zingiberaceae) based on morphological characters and isozyme pattern. In: Chantaranothai P (ed). *Proceeding of the 3rd Symposium on the Family Zingiberaceae*, Khon Kaen, Thailand.
- Crawford DJ. 1990. *Plant Molecular Systematics, Macromolecular Approaches*. John Wiley and Sons, New York.
- Deng JB, CB Ding, L Zhang, YH Zhou, Yang RW. 2011. Relationships among six herbal species (*Curcuma*) assessed by four isozymes. *Pyton* 80: 181-188
- Duke JA, Bogenschutz-Godwin JC, Duke PAK. 2002. *Handbook of Medicinal Herbs*. CRC Press, Boca Raton, FL.
- Feng X, Jiang ZT, Wang Y, Li R. 2011. Composition comparison of essential oils extracted by hydrodistillation and microwave-assisted hydrodistillation from *Amomum kravanh* and *Amomum compactum*. *J. Essen Oil-bearing Pl* 14 (3): 354-359.
- Garcia-Vallve S, Palau J, Romeu A. 1999. Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in *Escherichia coli* and *Bacillus subtilis*. *Mol Biol Evol* 9: 1125-1134.
- Garcia-Vallvé S, Puigbo P. 2002. DendroUPGMA: A dendrogram construction utility. *Biochemistry and Biotechnology Department*. Universitat Rovira i Virgili (URV). Tarragona, Spain. <http://genomes.urv.cat/UPGMA/>
- Guenther E. 1952. *The essential oils*. Vol. 5. Van Nostrand Co., New York.
- Hegnauer, R. 1963. *Chemotaxonomie der Pflanzen (Monocotyledoneae)*. Band II. Bessel: Birkhauser Verlag.

- Heyne K. 1950. De Nuttige Planten van Indonesie. Deel I. W. van Hoeve, 's-Gravenhage.
- Ibrahim H, Hussin KH, Sanusi A. 1991. Taxonomic implications of isozyme and anatomical studies on *Cucurma* species. Botany 2000 Asia. Zingiberaceae workshop, Prince of Songkla University, Hat Yai, Thailand, 15-18 October 1991.
- Ibrahim H. 1996. Isozyme variations in selected Zingiberaceae spp. In: Wu TL et al. (eds). Proceedings of the 2nd Symposium on the Family Zingiberaceae. Zhongshan University Press, Guangzhou, China.
- Jaccard P. 1908. Nouvelles recherches sur la distribution florale. Bull Soc Vard Sci Nat 44: 223-270
- Jia XP, Sun H. 2013. An intrinsic advantage of sexual reproduction. arXiv:1306.5373 (06/2013). 2013arXiv1306.5373J
- Jugran A, Bhatt ID, Rawat S, Giri L, Rawal RS, Dhar U. 2011. Genetic diversity and differentiation in *Hedychium spicatum* Buch. Ham. ex. D. Don - a high value medicinal plant of Indian Himalaya. Biochem Genet (DOI 10.1007/s10528-011-9451-7)
- Kasahara S, Hemmi S (eds.). 1995. Medicinal Herb Index In Indonesia, 2nd ed. PT. Eisai Indonesia, Jakarta.
- Kuyumcusavan E, Kucukbay FZ. 2013. Essential oil composition of *Elettaria cardamomum* Maton. J Appl Biol Sci 7 (3): 42-45, 2013.
- Madjo-Indo ABD. 1989. Kapulaga: Budidaya, Pengolahan dan Pemasaran. Penebar Swadaya, Jakarta. [Indonesian]
- Marongiu B, Piras A, Porcedda S. 2004. Comparative analysis of the oil and supercritical CO₂ extract of *Elettaria cardamomum* (L.) Maton. J Agric Food Chem 52 (20): 6278-6282
- Paisooksantivatana Y, Kako S, Seko H. 2001. Genetic diversity of *Curcuma alismatifolia* Gagnep. (Zingiberaceae) in Thailand as revealed by allozyme polymorphism. Genet Res Crop Evol 48 (5): 459-465.
- Peter KV. 2001. Handbook of Herbs and Spices. Vol. 1. CRC Press, New York.
- Purseglove JW, Brown EG, Green CL, Robbins SRJ. 1981. Spices, Volume 2. Longman Tropical Agriculture Series, London.
- Purseglove JW. 1972. Tropical Crops Monocotyledones. Longman, London.
- Santoso HB. 1988. Kapulaga. Penerbit Kanisius, Yogyakarta [Indonesian]
- Seidemann J. 2005. World Spice Plants: Economic Usage, Botany, Taxonomy. Springer, New York.
- Setyawan AD, Wiryanto, Suranto, Bermawie N. 2014. Variation in isozymic pattern of three varieties of ginger (*Zingiber officinale*) germplasm. Nus Biosci 6: 00-00
- Setyawan AD. 2002. Chemotaxonomic studies on the genus *Amomum* based on chemical components of volatile oils. Hayati J Biosci 9 (3): 71-79.
- Shamina A, Zacharias JT, Sasikumar B, George JK. 1998. Biochemical variation in turmeric (*Curcuma longa* Linn.) accessions based on isozyme polymorphism. J Horticult Sci Biotechnol 73 (4): 479-483.
- Shamita A, Zachariah TJ, Sasikumar B, George JK. 1997. Biochemical variability in selected ginger (*Zingiber officinale* Rosc.) germplasm accessions. J Spic Arom Crops 6 (2): 119-127.
- Shanmugapriya M, Prabha ML. 2012. Isoenzyme analysis of *Amomum* species. Intl J Pharm Biol Arch 3 (5): 1091-1094
- Sneath PHA, Sokal RR. 1973. Numerical Taxonomy. W.H. Freeman, Co., San Francisco.
- Suranto. 1991. Studies of Population Variations in Species of Ranunculus. [M.Sc.-Thesis]. Departement of Plant Science University of Tasmania, Hobart.
- Tang JY, Li QM, Yang RW, Liao JQ, Zhou YH. 2008. Study on isozymes in six species of *Curcuma*. China J Chin Mat Med 33 (12): 1381-1386.
- Trease GE, Evans WC. 1978. Pharmacognasy. 11thed. Bailliere Tindall, London.
- Vanijajiva O, Suvachittanont W, Sirirugs P. 2003. Isozyme analysis of relationships among *Boesenbergia* (Zingiberaceae) and related genera in Southern Thailand. Biochem Syst Ecol 31 (5): 499-511.
- Wardini TH, Thomas A. 1999. *Elettaria cardamomum* (L.) Maton. In: de Guzman CC, Siemonsma JS (eds.). Plant Resources of South-East Asia 13: Spices. PROSEA, Bogor.
- Weeden VF, Wendel JF. 1989. Visualization and interpretation of plant isozymes. In: Soltish DE, Soltish PS (eds.) Isozyme in Plant Biology. Dioscorides Press Portland, Oregon.
- Wolff XY, Hartutiningsih. 1999. *Amomum compactum* Soland. ex Maton. In: de Guzman CC, Siemonsma JS (eds.). Plant Resources of South-East Asia 13: Spices. PROSEA, Bogor.
- Youngken HW. 1948. Textbook of Pharmacognosy. 7th ed. The Blakiston Co., Philadelphia, Penn.
- Yu JG, Feng HJ, Li JT. 1982. Essential oil of fruits and leaves of *A. kravanh* and *A. compactum*. Chin Trad Herb Drugs 13: 4-7.