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

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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.

Keywords: *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* (Korarima 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; Wollf 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, Kulonprogro 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 literature stated that volatile oil of true cardamom is 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.

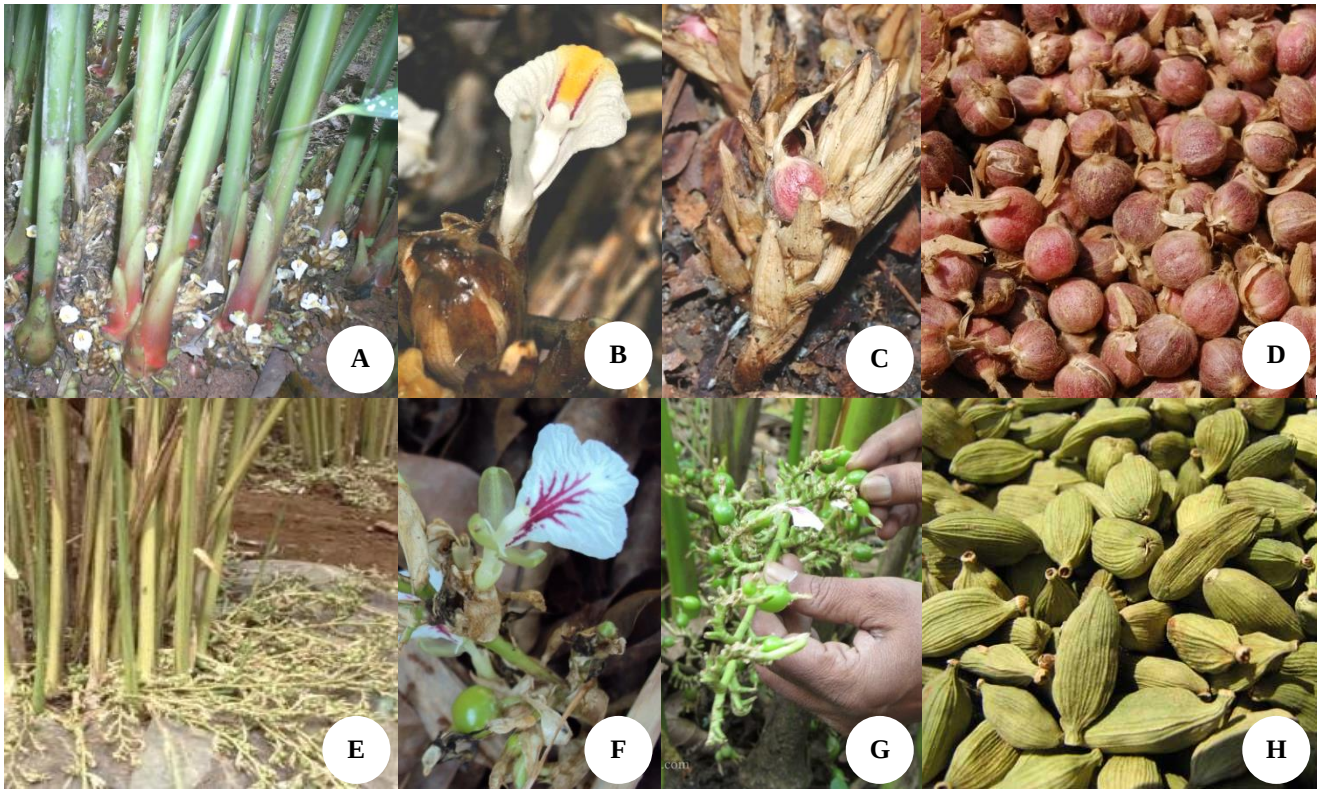

Figure 1. The morphology of *A. compactum*: A. Clumps, B. Flower, C. Fruits, D. Seeds (pods); and *E. cardamomum*: E. Clumps, F. Flower, G. Fruits, H. Seeds (pods). (photos from many sources)

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-terpinene, 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 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).

Researchofcardamomsvariabilityusingisozymic pattern has not been done yet, except for *Amomum aromaticum*, *A. cannicarpum*, *A. kingii*, and *A. subulatum* (Shanmugapriya andPrabha2012), while the same research on other gingers family had been conducted to *Boesenbergia*(Vanijajiva et al. 2003), *Curcumaalismatifolia*(Paisooksantivatana 2001), *C. aeruginosa*, *C. elata*, *C. rubescens*, *C. zedoaria* (Apavatjrut et al. 1999),*C. chuanhuangjiang*, *C. kwangsiensis*, *C. phaeocaulis*, *C. wenyujin* (Tang et al. 2008; Deng et al. 2011), *C. sichuanenesis* (Deng et al. 2011), *C.longa*(Shamina et al. 1998; Deng et al. 2011), *C.xanthorriza*(Apavatjrut et al. 1999; Azizah 2011), *Hedychiumspicatum*(Jugran et al. 2011), *Zingiber officinale* (Shamita1997; Setyawan et al. 2014 in this issue), and a few other species of *Curcuma* (ChokthaweepanichandPaisooksantivatana2003) 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 patterns of esterase and peroxidase, and (ii) the genetic relationship between two species based on the isozyme 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, 10accessions were assayed. A series of observations were 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 electrophoresisprocedurerefers toCrawford(1990) andWeedenandWendel(1989) as modified bySuranto(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 2-5mm. Shoots were cut and immediately used for examination, or were stored in a refrigerator at 4°Cformaximum 14days, but can only be used effectively within seven days after cutting. Leaf extracts that stored in a refrigerator at4°Ccansurvivefor 30days.

Buffer. Tank buffer was made by dissolving14.4g of boric acid and 31.5g of borax (sodium borate), in distilled water to a volume of 2L. Extraction buffer was made by dissolving0.018g of cysteine, 0.021g of ascorbic acid, and 5g of sucrose (PA) in 20 mL of borax buffer at pH8.4. Running buffer wasTAE(Tris-Acetic Acid-EDTA) 50xdilutedto a concentration of1x.

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 uL of glycerol and 50 uL bromphenol blue (BPB) dissolved in 200 uL 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 uL of TEMED, and 10 uL 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 uL TEMED, and 10 uL 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. Freshleaftissuewasput in the extraction buffer, with a ratio of 1:4 (w/v), i.e., 68 ug (0.068 g) of leaf samples were pulverizedin272 uL (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 at8500rpm for20minutes at 4°C, then soaked in ice crystals. Supernatant was put in the wells of gel.

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

Staining. *Peroxidase*: 0.0125g of O-Dianisidine was put into Erlenmeyer and dissolved with 2.5 mL of acetone, and then was added with 50 mL of0.2Macetate bufferpH4.5and2drops ofH_2O_2. *Esterase:*0.0125g of α-Naphthyl acetate was put into Erlenmeyer and dissolved with 2.5 mL of acetone, and then was added with 50 mL of0.2MphosphatebufferpH 6.5and0.0125gFastBlueBBSalt. Separately, gel was soakedto

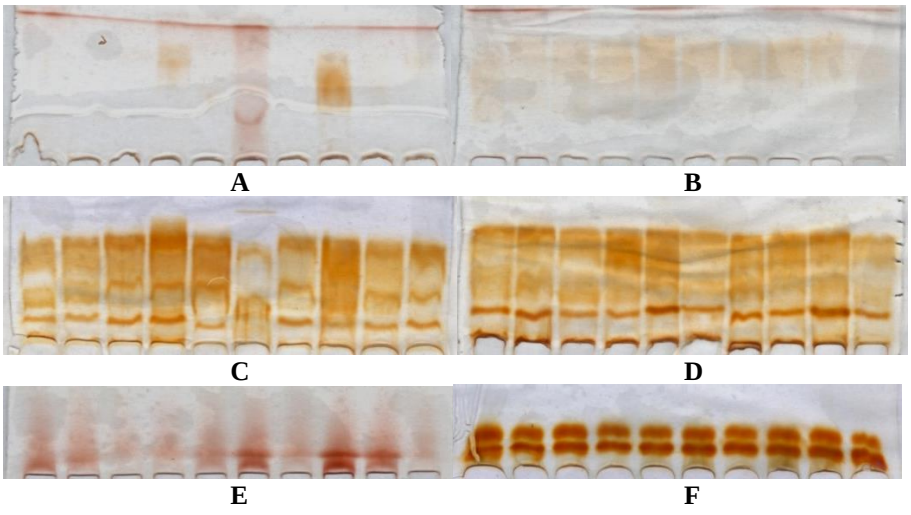

Figure 2. Zymogram of cardamoms. Java cardamom (A) and true cardamom (B) stained with esterase. Java cardamom (C) and true cardamom (D) stained with esterase. Java cardamom of B2P2TOOT Tawangmangu stained with esterase (E) and peroxidase (F).

those solutions for 10minutes and was shakengentlyevery2minutes. Once the banding pattern appeared, the enzyme dye 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 a 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.

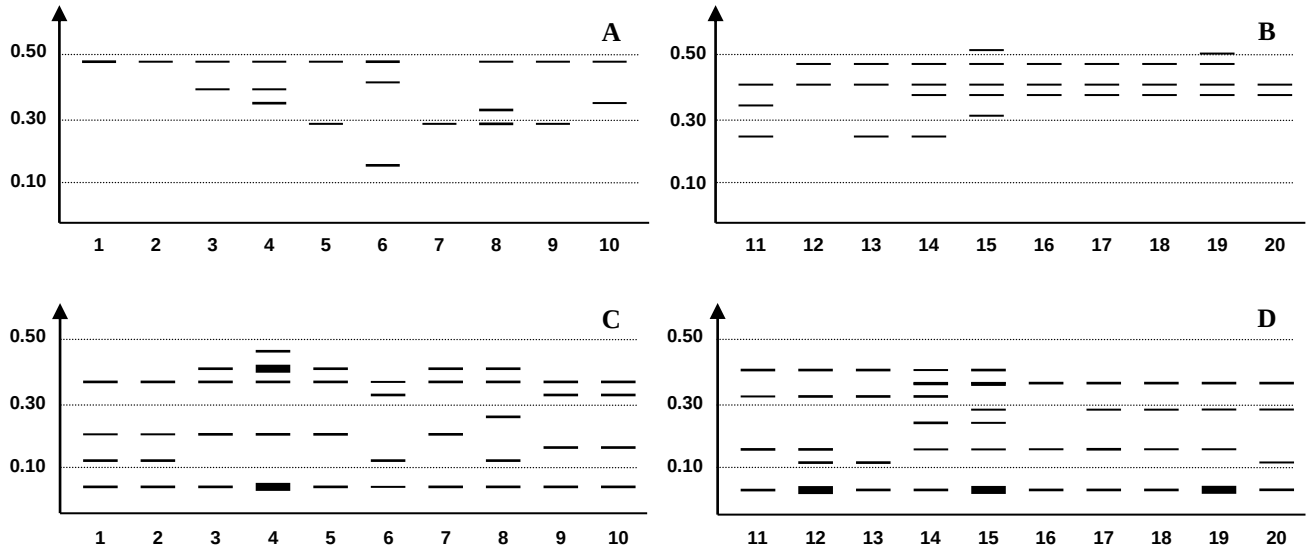

Figure 3. Schematic zymogram of cardamoms. Esterase isozymic pattern of Java cardamom (A) and true cardamom (B). Peroxidase isozymic pattern of Java cardamom (C) and true cardamom (D).

The number of esterase bands that emerged in this research is 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* is also only producing 1-4 isozymic bands depend on the species (Apavatjrut 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 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 result was 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 on samples from Java cardamomum 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 characters in the preparation dendrogram. Another 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 accession11 of Java cardamom and the accession16 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 generated 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 produces more isozymic bands than esterase does. 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 emerges itself (11). Based on zymogram, accession11 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 are two groups 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) is still mixed with 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 consists of only Java cardamomum (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. Matrix 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 esterase banding 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. Matrix 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 esterase and 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.

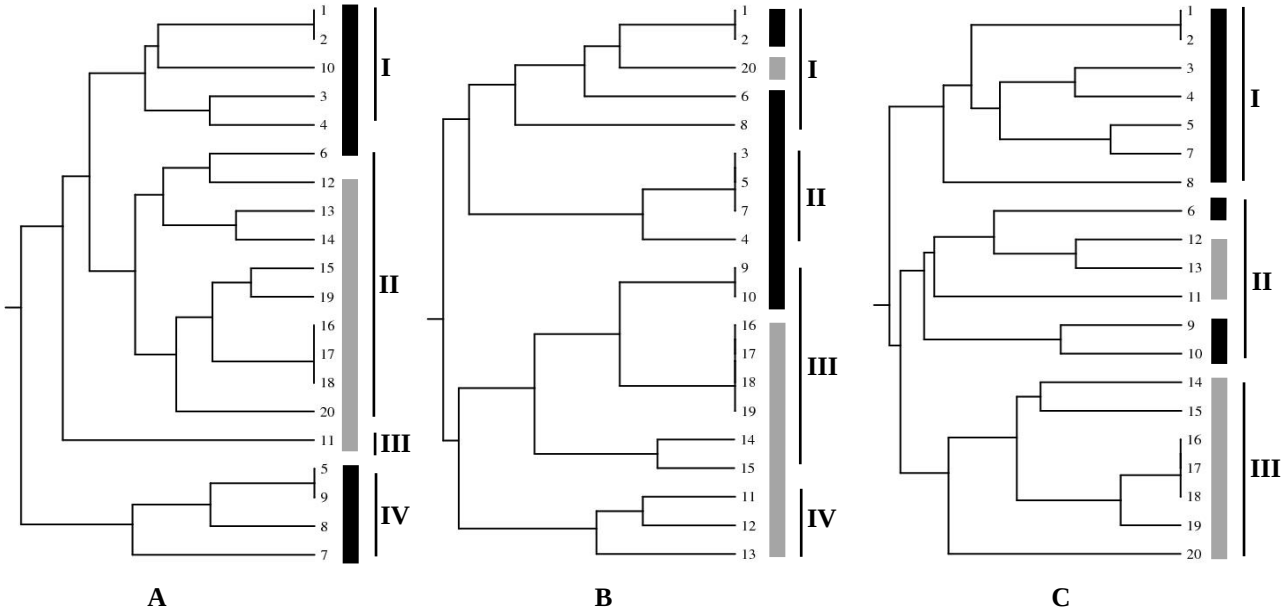

Figure 4. Relationship dendrogram of Java cardamom and true cardamom based on isozymicbanding pattern of esterase (A), peroxidase (B) and combinationofesterase andperoxidase (C). Note: ■ = Java cardamom, ■ = true cardamom.

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.

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