NUSANTARA BIOSCIENCE
Vol. 6, No. 1, pp. 86-93
May 2014

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n060114

Short Communication: Variation in isozymic pattern of germplasm from three ginger (*Zingiber officinale*) varieties

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Manuscript received: 10 March 2014. Revision accepted: 27 April 2014.

Abstract. *Setyawan AD, Wiryanto, Suranto, Bermawie N. 2014. Variation in isozymic pattern of germplasm from three of ginger* (Zingiber officinale) *varieties. Nusantara Bioscience 6: 86-93.* Ginger (*Zingiber officinale* Rosc.) has long been as spices, flavoring agent and raw material for herbal medicines. In Indonesia, there were three varieties based on color and size of the rhizome, i.e., gajah (big-white ginger), merah (red ginger), and emprit (small-white ginger). This research was conducted to find out: (i) isozymic pattern of three ginger varieties, and (ii) phylogenetic relationship of those three varieties. The plant materials were gathered from Wonogiri, Surakarta and Kulonprogo, Yogyakarta. Two enzyme systems, namely esterase (EST) and peroxidase (PER, PRX) were used in this study. The relationship among ginger varieties was determined by UPGMA. The result indicated that EST showed two bands (i.e. Rf 0.04 and 0.10), and PRX showed six bands (i.e. Rf 0.04, 0.05, 0.09, 0.10, 0.11, and 0.15). Peroxidase produces more numerous and more diverse isozymic bands than esterase, resulting in a more complex relationship. The data used to compile dendrogram affect the grouping; the more data used, the more obvious clustering of accessions in a population. Dendrogram generated from esterase and peroxidase banding patterns produced distinct clusters based on varieties and location.

Keywords: Ginger, isozyme, varieties, *Zingiber officinale,* Zingiberaceae

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), is one of the most important and oldest spices a well-known plant in the Nusantara archipelago, consumed as a delicacy, food preservation, medicine, or spice (de Padua et al. 1999). Its highest value is given by the pungent and aromatic essential oils produced throughout the plant, especially the rhizomes. The essential oils are responsible for the aroma while the non-volatile components are responsible for the pungency with gingerol the most pungent component in fresh ginger (Juliani et al. 2007). This herbaceous rhizomatous perennial reed-like plant with annual leafy stems is most useful Zingiberaceae in the tropics, as well as turmeric, cardamom and galangal (Poth and Sauer 2000).

The Zingiberaceaeconsist of approximately 47 genera and 1400 species, that are found in all tropical regions, but are mostly concentrated in Southeast Asia (Purseglove 1972; Kress 1990; de Padua et al. 1999). This spice is produced from the rhizome (underground stem) of the plant (Purseglove 1981). Distribution and use of ginger in Indonesia are very widespread (Burkill1935; Heyne1950). The aromatic and spicy rhizome is used as a spice, seasoning, and medicine (Heyne1950; Holttum 1950), and the less spicy rhizome was used for food and beverages.

Zingiber officinale has three varieties based on size and color of rhizome, i.e. *Z. officinale* var. *officinale* (big white ginger or giant ginger, *badak* or *gajah*), Z. *officinale* var. *amarum* (small white ginger, *emprit*), and *Z. officinale* var. *rubrum* (small red ginger, *merah* or *berem*) (Ochse 1931; Burkill 1935; Heyne 1950). These three varieties may partly be deferred from their essential oil contents and are used for different purposes. The essential oil content of the big white ginger is the lowest compared with the other varieties (Setyawan 2002). The big white ginger is usually used for fresh (green) ginger products, food, and beverages, while the other gingers are mostly used for medicinal purposes.

Ginger produces clusters of white and pink flower buds that bloom into yellow flowers (Watt and Breyer-Brandwijk 1962). There are no differences in flower characters between varieties of gingers; therefore it is necessary to use other characters. In agriculture, the proper identification and characterization of varieties of the cultivated plant are needed to obtain the right herbal material used to develop products of high economic value. It is also necessary for plant breeding as marker-assisted selection. Genetic markers are needed to trace the genetic nature of the parent and its descendants.

The assessment of genetic variation may be done by using various techniques such as isozymes or DNA analysis (Mondini et al. 2009). Isozymic pattern is a highly effective genetic marker that can be obtained through the quick and easy laboratory process, and less expensive than the DNA sequences. It can be used to determine genetic variation of populations, even to accession genetic differences (Crawford 1990). Isozyme can be used as a genetic trait to study the diversity of accessions in a population, classification of plant species, varieties and identifying of hybrid (Beer et al. 1993; Murphy and

Phillips 1993), as well as markers of plant resistance to certain diseases (Alcazar et al. 1995).

Isozyme is a codominant character; heterozygous accessions can be distinguished with homozygous (McDonald and McDermont 1993). However, the isozyme patterns may be affected by different environments and the stage of plant development. Moreover, only limited numbers of isozymes loci are available for certain taxa (McDonald and McDermont 1993; Mangolin et al. 1997; Garkava et al. 2000). Isozyme variations only reflect difference in protein-coding sequences or intron (Adam 1999; Sharma and Jana 2002).

Research on ginger diversity by using isozymic pattern has been done by Shamita (1997) irrespective of varieties, while the same research on the other Zingiberaceae family has been conducted on *Curcuma* (Ibrahim et al. 1991; Apavatjrut et al. 1999; Chokthaweepanich and Paisooksantivatana 2003; Tang et al. 2008; Deng et al. 2011), *Curcuma alismatifolia* (Paisooksantivatana 2001), *Curcuma longa* (Shamina et al. 1998), *Curcuma xanthorrhiza* (Azizah 2011), *Amomum* (Shanmugapriya and Prabha2012), *Boesenbergia* (Vanijajiva et al. 2003), *Hedychium spicatum* (Jugran et al. 2011) and several species of Zingiberaceae (Ibrahim et al. 1996).

The aim of this study was to determine (i) the variation of isozymic pattern of ginger (*Zingiber officinale* Rosc.) from three different varieties from two different populations, Wonogiri and Kulonprogo, Indonesia, differentiated by color of rhizomal peels and size (small red, small white, and big white) and (ii) the genetic relationship between populations based isozymic pattern.

Materials and methods

Plants material. Three different varieties of ginger (*Zingiber officinale* Rosc.), i.e. big white, small white, and small red gingers (Figure 1), were collected from the dry fields in Eromoko, Wonogiri, Central Java and Kokap, Kulonprogo, Yogyakarta, Indonesia, each location with three varieties and for each variety three accessions were collected. The rhizome was harvested at the end of the dry season at the age of about 9-10 months and stored for 2-3 months before investigated. The plant materials were authenticated at the Department of Biology, Sebelas Maret University, Surakarta, Indonesia. Enzyme dye 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), and modified by Suranto (1991).

Planting of rhizomes. The rhizome was placed in a plastic tray lined with wet paper or cloth to keep moisture, until the leavesgrowalong2-5mm. Shots were cut and immediately used for examining, or could be stored in a refrigerator at 4°C for maximum 14 days, but effectively used 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 14.4g of boric acid and 31.5g of borax (sodium borate), in distilled water to a volume of 2L. 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 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 sodium dodecyl sulfate (SDS) dissolved in 120 mL of distilled water, adjusted to pH 8.8 by adding HCl, then added 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 dissolved in 400 mL of distilled water and then makeup 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 1st stock solution and 5.25 mL of the 2nd stock solution, added to 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 2nd stock solution and 1.15 mL of the 3rd stock solution, added to 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 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.

Figure 1. Rhizomeofgingervarieties. A.Small red ginger, B.Small white ginger, C. Big white ginger. (photo: Anna Frodesiak)

Extraction. Fresh leaf tissue was put in the extraction buffer, with a ratio of 1:4 (w/v), i.e., 68 ug (0.068 g) of leaf samples were pulverized in 272 uL (0.272 mL) of extraction buffer. Crushed in a porcelain dish that was placed above ice crystals, to keep cold (4°C). Samples were centrifuged at 8500rpm for20minat 4°C, then soaked in ice crystals. Supernatant was put in the wells of the gel.

Electrophoresis. 3.5 uL supernatant was pipetted with a micropipette, added with loading dye and sample loading guide, and then placed in the wells. The electrophoresis was undertaken at 200 volts, 60 mA for 5 minutes to reach the separating gel, and followed by further electrophoresis at 150V, 400 mA, for 60 minutes, until the loading dye reaches ~56 mm from the wells toward anode. The gel was transferred into a plastic tray and colored with enzyme dyes.

Staining. *Peroxidase*: 0.0125g of O-Dianisidine put into Erlenmeyer and dissolved to 2.5 mL of acetone, and then added to 50 mL of 0.2M acetate buffer pH 4.5 and two drops of H_2O_2. *Extras:* 0.0125g of α-Naphthyl acetate put into Erlenmeyer and dissolved in 2.5 mL of acetone, and then added to 50 mL of 0.2M phosphate buffer pH 6.5 and 0.0125 g Fast Blue BB Salt. Separately, the gel was soaked into that solution for 10 minutes and shaken gently every 2 minutes. Once the banding pattern appears, the enzyme dye was disposed and rinsed with distilled water, then recorded by a camera or scanner.

Data analysis. Data were analyzed qualitatively based on the presence or absence of bands. Only clear, unambiguous and reproducible bands were considered for data analysis. Rf (*retardation factor*) value was calculated based on the relative movement of isozyme and loading dye. The present bands were given a value of 1, while those absence bands were given a value of 0. Data were entered in a spreadsheet to create a binary matrix. The genetic similarity and genetic distance among the accessions were calculated according to Jaccard coefficients (Jaccard 1908). The matrices were computed and the corresponding dendrograms of genetic relatedness were constructed by applying the unweighted 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

Variation of isozymic pattern

Ginger samples used in this study had a relatively uniform condition; they were all harvested at maturity (9-10 months), stored for 2-3 months, and then planted to get the shoot tip. Uniformity stage of plant development is very important to ensure similar types of enzymes produced. Physiological developmental stages of an accession can produce different types of enzymes, as observed in the stages of embryogenesis (Bapat et al. 1992; Rout and Das 1995; Dodeman and Ducreux 1996), fruit development (Loveless 1992; Sadka et al. 2000), and colorizing plants (Barrett and Shore 1989). Isozyme pattern may also change in response to environmental condition, which followed by genetic material changes (Kahler et al. 1980; Mastenbroek et al. 1984; Guse et al. 1988; El-Bendary et al. 1998; Li Z and Rutger 2000; Gämperle and Schneller 2002; Oja 2002; Staszak et al. 2007; Zhang et al. 2009; Dasgupta et al. 2010; Shah and Nahakpam 2012). Figure 2 showed examples of zymogram that demonstrate the diversity of ginger by esterase and peroxidase banding pattern.

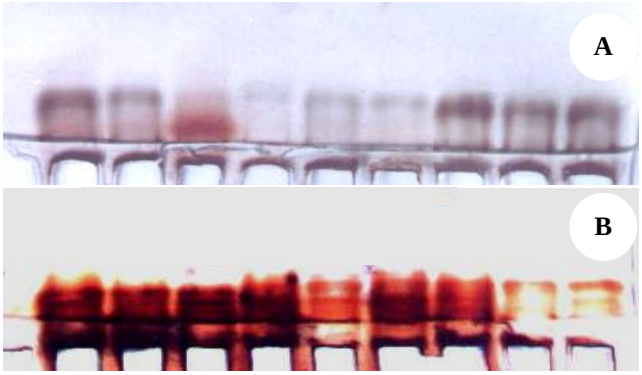

Figure 2. Zymogram of ginger using enzyme staining systems of esterase (A) and peroxidase (B).

Esterase (EST). Esterases (EC no. 3.1.x) are hydrolyzing enzymes which catalyze the addition or removal of water in biological reactions by splitting esters into acids and alcohols in a chemical reaction with water that called hydrolysis. Variation in esterases banding patterns exists due to differences in substrate specificity, protein structure, and biological function. Wide variation in esterases are reported in nature and occurs frequently in plants (IUBMB 1992).

In this observation, there were not many variations of esterase banding pattern among different ginger cultivars. Esterase isozyme indicated only two bands EST-1 located at Rf 0.10 and EST-2 located at Rf 0.04 from anodal zone, with purple-blue to red color (Figure 3.A). Some bands are fairly thick, but there are also thin bands or very thin, thus overlooked in the research. The difference of isozyme banding thickness is probably due to the differences in the copy number of the gene. On the other hand, a thick band may also be caused by two bands coincide, which indicates heterozygote for two alleles of the monomer, and a thin band indicating homozygote, however, esterase generally have thick bands.

The combination of the presence esterase isozymic banding produced four variations of the 18th accessions tested. In accession 3, small red ginger of Wonogiri, a band of EST-1 at Rf 0.04 was very thick, while in the other accessions was very thin or absent. The existence of unique band was very valuable as a marker for identification of the accession and useful in conservation biology. Perhaps the difference in staining intensity may be related to certain agronomic important traits, but this must be confirmed.

On the other ginger family, esterases are also generating a little variation in isozymic banding patterns. Application of esterase on some species of *Curcuma* only produced 1-4 bands depending on the species (Apavatjrut et al. 1999), while in some species of *Amomum* this enzyme raised only one band, and could not show the variation among species (Shanmugapriya and Prabha 2012). The limited number of banding patterns caused low variation among populations.

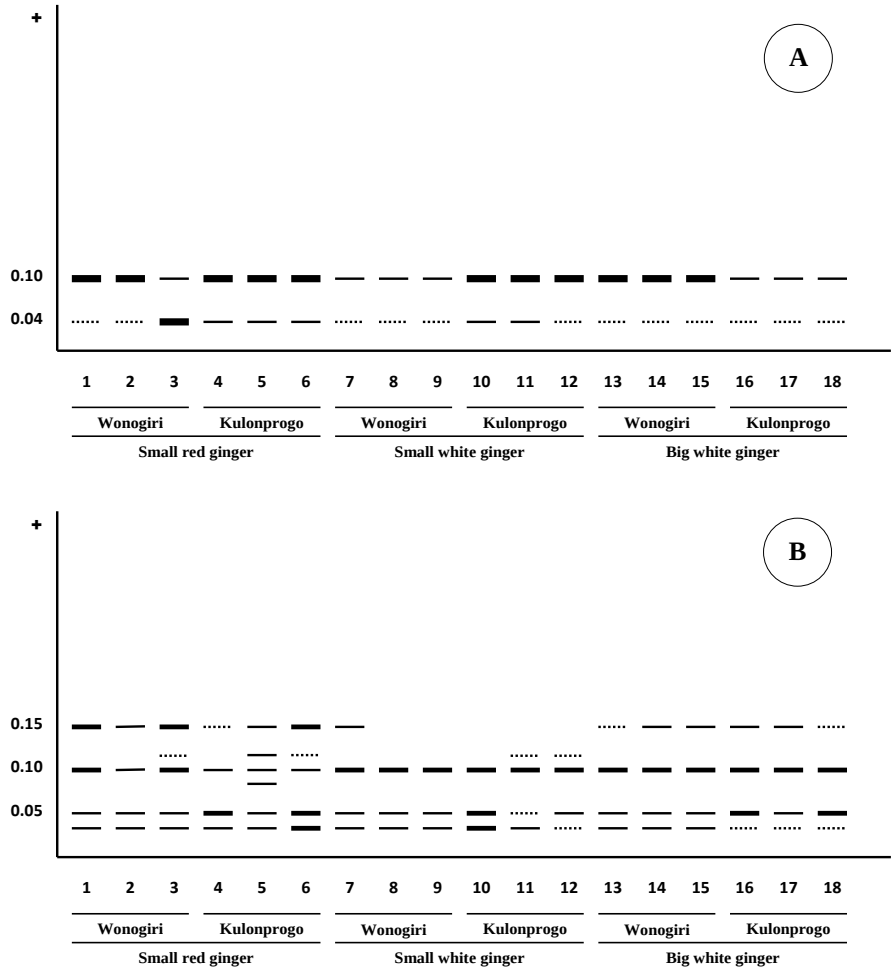

Figure 3. Isozymic banding pattern of ginger varieties. A. Esterase, B. Peroxidase. Note: 1,2,3. Small red ginger of Wonogiri; 4,5,6. Small red ginger of Kulonprogo; 7,8,9 Small white ginger of Wonogiri; 10,11,12. Small red ginger of Kulonprogo; 13,14,15. Big white ginger of Wonogiri; 16,17,18. Big white ginger of Kulonprogo.

The results showed that among the three accessions in one population (the same varieties and locations), two or all three have in common esterase isozymic pattern, but the same pattern can also be found precisely in accessions of different varieties and locations. It was shown that there was ginger uniting each other based on the location, i.e., Wonogiri and Kulonprogo or on the variety, i.e., big white ginger, small white ginger, and small red ginger. But this tendency was disguised by grouping of different location and a variety. A small number of band pattern emerged and it supported this complication. This is most likely because it was taken from the same location, and ginger is always propagated vegetatively thus genetically similar. It might be caused by the history of evolution of the three gingers. They were made up divergently, thus they changed each other genetic code, or the emerging morphological difference might be being coded by genetic material, which has no relation with esterase enzyme.

Peroxidase (PER, PRX). Peroxidases (EC no. 1.11.1.x) are a large family of enzymes that typically catalyze a reaction of ROOR' + electron donor (2 e-) + 2H+ → ROH + R'OH. For many of these enzymes the optimal substrate is hydrogen peroxide, but others are more active with organic hydroperoxides such as lipid peroxides. Peroxidases can contain a heme cofactor in their active sites, or alternately redox-active cysteine or seleno-cysteine residues (IUBMB 1992).

In this study, peroxidase indicated six isozymic bands, PRX-1 at Rf 0.04, PRX-2 at Rf 0.05, PRX-3 at Rf 0.09, PRX-4 at 0.10, PRX-5 at Rf 0.11, and PRX-6 at Rf 0.15 with red color (Figure 3.B). Of the 18 accessions tested, as many as 8 variations of the isozyme banding pattern appear; hence the character is very valuable as distinguishing evidence. Two unique bands were found in accession number 5 (small red ginger of Kulonprogo) at Rf 0.09, and 0.11. The band at Rf 0.9 was only found in accession number 5, while the band at Rf 0.11 was also detected in accession number 3, 6 (small red ginger), 11 and 12 (small white ginger). These bands were not detected in the other accessions. The band at Rf 0.9 can be used as genetic markers for accession 3. Peroxidase created many variations of isozymic banding patterns. In higher plants, peroxidase has a wide distribution and exhibit broad substrate specificity. On another ginger family, namely *Amomum*, peroxides are generating four banding patterns. It is much more than esterase that creating one band only (Shanmugapriya and Prabha 2012).

The presence of many peroxides patterns caused more varied relationship. The isozyme can be used to test genetic variability between accessions within a species. It caused accession of the same population, i.e., similar in variety and planting location, able to have a different isozymic pattern, thus the relationship would be in a different situation, even though there could be a tendency that the same accessions of the same population tend to have a closer relationship than accession of different population.

Phylogenetic relationship

Esterase (EST). The esterase only generated two isozymic bands at Rf 0.04 and 0.10. Variations between populations are very little, only two clusters. In dendrogram, many accessions join at the similarity index up to 100%, although different varieties and locations of origin. Except for accession 3, all accessions of ginger of

Wonogiri, both small red ginger (1,2), small white ginger (7, 8, 9), and big white ginger (13,14,15), and also one accession of small white ginger of Kulonprogo (12) and big white ginger of Kulonprogo (16, 17, 18) join with similarity index up to 100%, although these populations are from different varieties and locations of origin.

Other accession joins a cluster with a similarity index of up to 100%, i.e., one small red ginger of Wonogiri (3), all accessions of small red ginger of Wonogiri (4,5,6) and two small white ginger of Kulonprogo (10, 11). In this cluster, some varieties the same accessions join, but a different site of origin, i.e., small red ginger of Wonogiri (3) and of Kulonprogro (4,5,6). There is also the accession of the same origin, but different varieties, i.e., all small red ginger (4,5,6) and two small white ginger (10,11) of Kulonprogo. However, ginger that derived from the same varieties and location generally have a very strong relationship with similarity index up to 100%, for example, all populations of the small red ginger of Kulonprogo (4, 5, 6), big white ginger of Wonogiri (13, 14, 15), small white ginger of Wonogiri (7, 8, 9), and big white ginger of Kulonprogo (16,17,18) (Table 1A; Figure 4.A).

The dendrogram showed that among the three accessions of ginger in one variety and one location, the two or all of them have a same esterase banding pattern up to 100%, but the same pattern could be found precisely with similarity index up to 100% on accession from different variety and location (Figure 4.A). Since, esterase only generate two isozymic banding patterns, many different varieties, and location of ginger joint together in a cluster, that less reflects the grouping based on variety and location.

Peroxidase (PER, PRX). The peroxidase isozyme generated six bands with Rf value of 0.04, 0.05, 0.09, 0.10, 0.11, and 0.15. Peroxidase has much more variation of isozymic pattern. Relationship dendrogram shows that there are only two independent ginger accessions, where the similarity index with the other accession less than 100%, i.e., the accession small red ginger of Kulonprogro (5) and the accession small white ginger of Kulonprogro (11). Meanwhile, other accessions have in similarity index up to 100% with one or more other accessions. There is a large cluster with similarity index 100% consisting of various varieties of ginger, i.e., three accessions small red ginger of Wonogiri (1, 2, 3) and one of Kulonprogo (6), one accessions small red ginger of Wonogiri (7) and two accessions small red ginger of Kulonprogo (14, 15).

There is also a cluster with % similarity index 100that contains one accession small red ginger of Kulonprogo (4), two accessions small white ginger of Wonogiri (8, 9), one accession small red ginger of Kulonprogo (10), and one accession big accession white ginger of Wonogiri (13). Meanwhile, two ginger accessions of Kulonprogo from different varieties have up to similarity index 100%, i.e. one accession small white ginger (12) and one accession of big white ginger (18). On the other hand, two big white ginger accessions of Kulonprogo (16, 17) form a separate cluster.

Those accessions are generally still joined in one group, although one of them is a separate accession. Population ginger that still collects all members in the group with similarity index 100% is the small red ginger of Wonogiri (1,2,3). Meanwhile, on the other population, there are only two accessions clustered together with similarity index 100%, while the others are separated, for example, small red ginger of Wonogiri (1,2, 3), small white ginger of Wonogiri (8,9), the big white ginger of Wonogiri (14,15), and big white ginger of Kulonprogo (16,17) (Table 1.B; Figure 4.B).

Grouping generally occurs in accessions that have same varieties and locations of origin, but it may also occur in accessions that have different varieties and locations of origin. This because, ginger generally propagated vegetatively and have close relationships, although differ on varieties or locations of origin. Meanwhile, the existence of stand-alone accessions, i.e., the accession small red ginger of Kulonprogro (5) and the accession small white ginger of Kulonprogro (11), shows the existence of evolutionary process, i.e. changes in the genetic composition of vegetative propagation of ginger. This mutation is important for survival against environmental changes.

Combination of esterase and peroxidase. The dendrogram, which based on the combination of esterase and peroxidase markers, is clearer in showing the grouping based on its variety, locations of origin or both. Grouping based on location can be found either in ginger population of Wonogiri or Kulonprogo. In Wonogiri, two accessions red gingers (1, 2), one accession small white gingers clustered with two accessions big white gingers (14, 15) with similarity index 100%. Meanwhile, two small red ginger accessions from different locations, i.e., Wonogiri (3) and Kulonprogo (6) is also clustered with the similarity index 100%. Finally, the two groups joined.

In Kulonprogo, the accession small white ginger (12) and big white ginger (18) which has similarity index 100%, and the two accessions big white ginger (16, 17) which also has similarity index 100% joined in one cluster. On the other hand, two accessions of different variety are also clustered with the similarity index 100%, i.e. small red ginger (4) and small white ginger (10). In Wonogiri, two accessions small white ginger (8, 9) and one accession big white ginger (13) also integrates with similarity index 100%. Finally, the two groups joined (Table 2; Figure 4.C).

Relationship dendrogram shows that the more data that is used as a distinguishing character, the clearer the grouping based on the variety and growing location. On esterase-based dendrogram, it appears that the groupings based on population are less reflected, while on peroxidase-based dendrogram, the presence of grouping in population is more obvious. In the dendrogram based on the combined data of esterase and peroxidase, the population with which the members fall into one group is increasing.

Meanwhile, as in peroxidase-based dendrogram, it is shown that in the population, the two members congregate but the other one apart. In the combined dendrogram accession 5 and 11, each of them tends to stand alone. The existence of such accessions is very valuable for plant breeding, because of these differences reflect a typical mutation, making it very useful in adapting to the climate and environment changes, such as global warming and land use change (Table 2).

Table 1. Matrix of similarity computed with Jaccard coefficient on three varieties of ginger from Wonogiri and Kulonprogo, Indonesia based on esterase and peroxidase isozymic pattern.

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
1	1	1.000	0.500	0.500	0.500	0.500	1.000	1.000	1.000	0.500	0.500	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	1.000	1	0.500	0.500	0.500	0.500	1.000	1.000	1.000	0.500	0.500	1.000	1.000	1.000	1.000	1.000	1.000	1.000
3	1.000	1.000	1	1.000	1.000	1.000	0.500	0.500	0.500	1.000	1.000	0.500	0.500	0.500	0.500	0.500	0.500	0.500
4	0.750	0.750	0.750	1	1.000	1.000	0.500	0.500	0.500	1.000	1.000	0.500	0.500	0.500	0.500	0.500	0.500	0.500
5	0.667	0.667	0.667	0.500	1	1.000	0.500	0.500	0.500	1.000	1.000	0.500	0.500	0.500	0.500	0.500	0.500	0.500
6	1.000	1.000	1.000	0.750	0.667	1	0.500	0.500	0.500	1.000	1.000	0.500	0.500	0.500	0.500	0.500	0.500	0.500
7	1.000	1.000	1.000	0.750	0.667	1.000	1	1.000	1.000	0.500	0.500	1.000	1.000	1.000	1.000	1.000	1.000	1.000
8	0.750	0.750	0.750	1.000	0.500	0.750	0.750	1	1.000	0.500	0.500	1.000	1.000	1.000	1.000	1.000	1.000	1.000
9	0.750	0.750	0.750	1.000	0.500	0.750	0.750	1.000	1	0.500	0.500	1.000	1.000	1.000	1.000	1.000	1.000	1.000
10	0.750	0.750	0.750	1.000	0.500	0.750	0.750	1.000	1.000	1	1.000	0.500	0.500	0.500	0.500	0.500	0.500	0.500
11	0.500	0.500	0.500	0.667	0.333	0.500	0.500	0.667	0.667	0.667	1	0.500	0.500	0.500	0.500	0.500	0.500	0.500
12	0.500	0.500	0.500	0.667	0.333	0.500	0.500	0.667	0.667	0.667	0.333	1	1.000	1.000	1.000	1.000	1.000	1.000
13	0.750	0.750	0.750	1.000	0.500	0.750	0.750	1.000	1.000	1.000	0.667	0.667	1	1.000	1.000	1.000	1.000	1.000
14	1.000	1.000	1.000	0.750	0.667	1.000	1.000	0.750	0.750	0.750	0.500	0.500	0.750	1	1.000	1.000	1.000	1.000
15	1.000	1.000	1.000	0.750	0.667	1.000	1.000	0.750	0.750	0.750	0.500	0.500	0.750	1.000	1	1.000	1.000	1.000
16	0.750	0.750	0.750	0.500	0.500	0.750	0.750	0.500	0.500	0.500	0.250	0.667	0.500	0.750	0.750	1	1.000	1.000
17	0.750	0.750	0.750	0.500	0.500	0.750	0.750	0.500	0.500	0.500	0.250	0.667	0.500	0.750	0.750	1.000	1	1.000
18	0.500	0.500	0.500	0.667	0.333	0.500	0.500	0.667	0.667	0.667	0.333	1.000	0.667	0.500	0.500	0.667	0.667	1

B. Similarity matrix of peroxidase banding pattern

Note: 1,2,3. Small red ginger of Wonogiri; 4,5,6. Small red ginger of Kulonprogo; 7,8,9 Small white ginger of Wonogiri; 10,11,12. Small white ginger of Kulonprogo; 13,14,15. Big white ginger of Wonogiri; 16,17,18. Big white ginger of Kulonprogo.

Table 2. Matrix of similarity and distance computed with Jaccard coefficient on three varieties of ginger from Wonogiri and Kulonprogo, Indonesia based on a combination of isozymic pattern of esterase and peroxidase.

Similarity matrix computed with Jaccard coefficient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1		1.000	0.833	0.667	0.625	0.833	1.000	0.800	0.800	0.667	0.500	0.600	0.800	1.000	1.000	0.800	0.800	0.600
2	0.000		0.833	0.667	0.625	0.833	1.000	0.800	0.800	0.667	0.500	0.600	0.800	1.000	1.000	0.800	0.800	0.600
3	0.167	0.167		0.833	0.750	1.000	0.833	0.667	0.667	0.833	0.667	0.500	0.667	0.833	0.833	0.667	0.667	0.500
4	0.333	0.333	0.167		0.625	0.833	0.667	0.800	0.800	1.000	0.800	0.600	0.800	0.667	0.667	0.500	0.500	0.600
5	0.375	0.375	0.250	0.375		0.750	0.625	0.500	0.500	0.625	0.500	0.375	0.500	0.625	0.625	0.500	0.500	0.375
6	0.167	0.167	0.000	0.167	0.250		0.833	0.667	0.667	0.833	0.667	0.500	0.667	0.833	0.833	0.667	0.667	0.500
7	0.000	0.000	0.167	0.333	0.375	0.167		0.800	0.800	0.667	0.500	0.600	0.800	1.000	1.000	0.800	0.800	0.600
8	0.200	0.200	0.333	0.200	0.500	0.333	0.200		1.000	0.800	0.600	0.750	1.000	0.800	0.800	0.600	0.600	0.750
9	0.200	0.200	0.333	0.200	0.500	0.333	0.200	0.000		0.800	0.600	0.750	1.000	0.800	0.800	0.600	0.600	0.750
10	0.333	0.333	0.167	0.000	0.375	0.167	0.333	0.200	0.200		0.800	0.600	0.800	0.667	0.667	0.500	0.500	0.600
11	0.500	0.500	0.333	0.200	0.500	0.333	0.500	0.400	0.400	0.200		0.400	0.600	0.500	0.500	0.333	0.333	0.400
12	0.400	0.400	0.500	0.400	0.625	0.500	0.400	0.250	0.250	0.400	0.600		0.750	0.600	0.600	0.750	0.750	1.000
13	0.200	0.200	0.333	0.200	0.500	0.333	0.200	0.000	0.000	0.200	0.400	0.250		0.800	0.800	0.600	0.600	0.750
14	0.000	0.000	0.167	0.333	0.375	0.167	0.000	0.200	0.200	0.333	0.500	0.400	0.200		1.000	0.800	0.800	0.600
15	0.000	0.000	0.167	0.333	0.375	0.167	0.000	0.200	0.200	0.333	0.500	0.400	0.200	0.000		0.800	0.800	0.600
16	0.200	0.200	0.333	0.500	0.500	0.333	0.200	0.400	0.400	0.500	0.667	0.250	0.400	0.200	0.200		1.000	0.750
17	0.200	0.200	0.333	0.500	0.500	0.333	0.200	0.400	0.400	0.500	0.667	0.250	0.400	0.200	0.200	0.000		0.750
18	0.400	0.400	0.500	0.400	0.625	0.500	0.400	0.250	0.250	0.400	0.600	0.000	0.250	0.400	0.400	0.250	0.250	

Distance matrix based on Jaccard coefficient

Note: 1,2,3. Small red ginger of Wonogiri; 4,5,6. Small red ginger of Kulonprogo; 7,8,9 Small white ginger of Wonogiri; 10,11,12. Small white ginger of Kulonprogo; 13,14,15. Big white ginger of Wonogiri; 16,17,18. Big white ginger of Kulonprogo.

This study still requires further study to obtain a more stable marker accuracy, but the identification result has been added the information on genetic diversity of ginger for the selection and for the improvement of varieties. Further research with other chromosomal data, such as karyotype and molecular cytogenetic will greatly assist the identification of ginger diversity, especially when they are combined with DNA sequence data.

Isozymes were only able to detect the genetic diversity in the introns, which was then translated into protein material. This led to the ability of isozyme, as distinguishing characteristics are relatively limited, given the parts of introns that are not translated are undetectable for its diversity. Some researchers began to leave isozymes as genetic markers and test its genetic diversity right on the DNA.

Results showed that ginger undergoes genetic variation due to a wide range of ecological conditions. This investigation was an understanding of genetic variation within the accessions. It will also provide an important input into determining resourceful management strategies and help to breeders for the ginger improvement program.

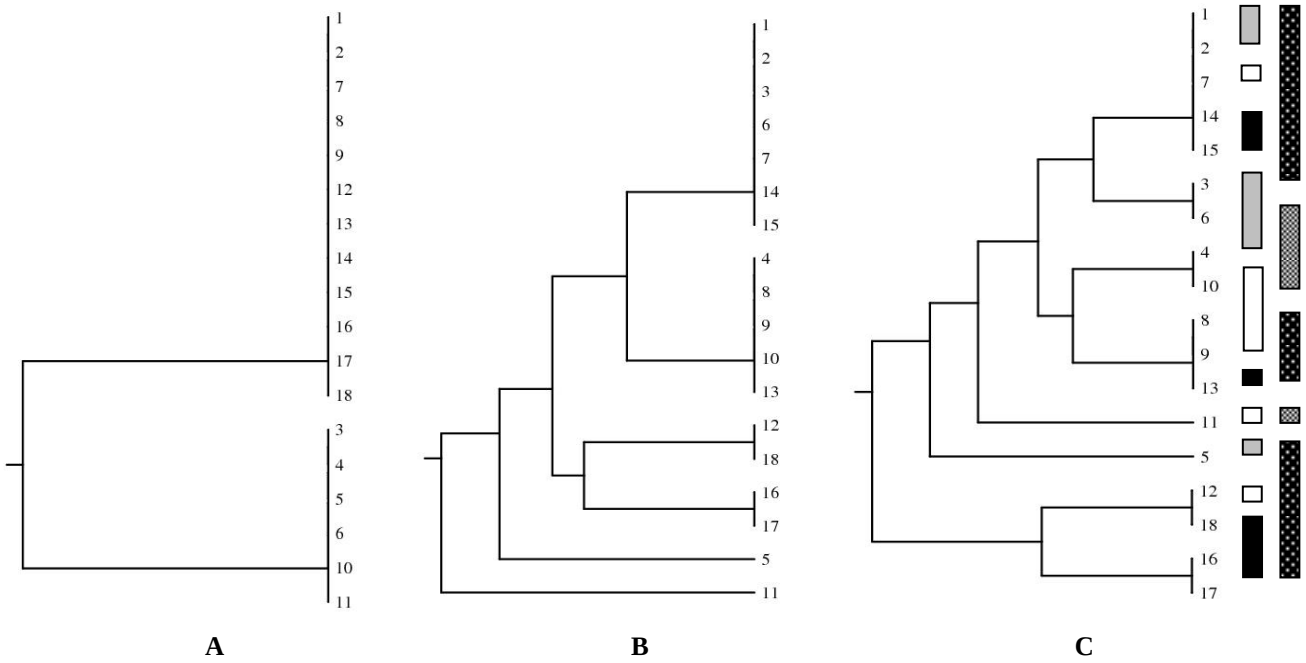

Figure 4. The dendrogram relationship of ginger varieties based on isozymic banding patterns of esterase (A), peroxidase (B) and combination of esterase and peroxidase (C). Note: 1,2,3. Small red ginger of Wonogiri; 4,5,6. Small red ginger of Kulonprogo; 7,8,9 Small white ginger of Wonogiri; 10,11,12. Small white ginger of Kulonprogo; 13,14,15. Big white ginger of Wonogiri; 16,17,18. Big white ginger of Kulonprogo. Note: ▭ small red ginger, □ small white ginger, ■ big white ginger; ▩ Kulonprogo ginger, ▨ Wonogiri ginger.

In conclusion, variation was observed in esterases and peroxidases in the three varieties of ginger. Esterase generated two isozyme bands, at the Rf value of 0.04 and 0.10, while peroxidase generated six isozyme bands, at the Rf value of 0.04, 0.05, 0.09, 0.10, 0.11, and 0.15. Accessions 3 has specific isozyme banding, on the other hand, accession 5 has the most specific peroxidase banding. Dendrogram on esterase generated 4 groups of 18 accessions; while based on peroxidase, there were 15 groups. Peroxidase produces more numerous and more diverse isozymic bands than esterase, resulting in a more complex relationship. The amount of data that is used to compile dendrogram affects the grouping, the more data used, the more obvious clustering of accessions in a population.

ACKNOWLEDGEMENTS

The part of the work was supported by the DGHE Republic of Indonesia with research grant no. 022/LIT/BPPK-SDM/IV/2002. The authors thank Dr. Molide Rizal of Balittro Bogor, Indonesia for valuable discussion on ginger diversity. The authors also thank Suhar Iriyanto and Delima Susanti Roesyat for his generosity in providing some plant materials, and Setyanto and Asriyati Asih Wardani for the kindly assistance with the laboratory work.

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