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DIVERSITY OF SOME INDONESIA LOCAL GLUTINOUS RICE (*Oryza sativa* L. Var. Glutinous) BASED ON AGROMORPHOLOGICAL AND RAPD MARKERS

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SUMMARY

Ketan is a term for glutinous rice in Indonesia that is widely used as an ingredient in various traditional dishes or desserts. However national production of this plant is low because most of the farmers prefer to cultivate paddy. This research aimed to investigate genetic diversity of potential parents. These results will be used to create new varieties of glutinous rice. The plant materials used in the experiments included eight local upland glutinous rice cultivars from West Java, Banten, and Bengkulu province. The seeds were planted by drill seeding in the experimental plots. The observations were recorded on quantitative and qualitative characters of each cultivar. The results showed that cv. Pisitan Bulu and cv. Ketan Hideung had high values for seed weight (above 3 g/100grain), with plant height around 180 cm, long maturity (97.5 DAP for 50% flowering), and low tiller number (less than 10 tillers/clump). These weaknesses can be overcome by cv. Hitam-46 and Kelapa (plant height < 120 cm, tiller number > 20 tillers/clump and 50% flowering around 75 until 80 DAP). Almost all cultivars were resistant to blast disease. The results suggested that Pisitan Bulu, Ketan Hideung and Ketan Hitam-46 could be used as potential as parents in breeding programs. The results of this research indicated that the use of RAPD markers combined with agromorphological traits are useful for identifying genetic diversity of glutinous rice. Based on the dendogram from genetic analysis, qualitative, and quantitative characters, Ketan Kelapa and Ketan Hitam-46 were similar even though the origin was different (Ketan Hitam-46 from West Java, Ketan Kelapa from Enggano).

Key words: Glutinous rice, agromorphological, genetic characters, upland

Key findings: This study investigated Indonesian local upland glutinous rice characters to evaluate their potential for use in breeding programs.

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INTRODUCTION

Glutinous rice is consumed in a main dish, but also used in different forms to make various kinds of food products such as dessert, snacks and toppings (Wiset *et al*., 2011). In Indonesia, the production of *ketan* grain yield is limited because the farmers are growing these varieties in small areas and they use local varieties with low productivity. There is a similar situation in Northeast Thailand (Naing *et al*., 2010). Based on Indonesian data, glutinous rice production and consumption has not been well recorded and is still generally categorized as part of total rice production data. Low attention to glutinous rice can lead to an extinction of genetic variability, especially for upland, glutinous varieties. Ethnographic studies suggest that glutinous rice cultivation is associated with upland agriculture in mainland Southeast Asia (Olsen and Purugganan, 2002). Some cultivars of glutinous rice with high yield produced by the Indonesian Ministry of Agriculture were potential cultivars for irrigated lowland areas, such as Lusi, IR65, Ketonggo, Ciasem, and Setail (black glutinous rice) (Santika and Rozakurniati, 2010).

The genetic variation of glutinous rice has been drastically reduced due to many factors such as national variety recommendations, low interest of farmers to grow these varieties, reductions in cultivated land, and low productivity. The development of new glutinous rice varieties is vital to increase productivity and quality. Genetically, local glutinous varieties have good quality characteristics, but generally longer time for maturity and have low productivity. Therefore, local glutinous rice resources need to be saved in gene banks for future varietal improvements (Ahmed *at al*., 2016). The brown glutinous rice in the market generally comes from local cultivars with long growth durations (5-6 months) and yields that are about 40-50% lower than recommended glutinous rice varieties (Santika and Rozakurniati, 2010).

Plant phenotype is the result of interactions between genetic and environmental factors. If the trait phenotype is more due to genetic factors rather than the environment, it will facilitate the process of plant selection during the breeding process. The potential productivity of upland rice in India could increase with a combination of improved cultivars and agronomic management (Mandal *et al*., 2010). Molecular markers have been widely used in plant characterization and analysis of genetic diversity (Kristamtini *et al*., 2014; Lestari *et al.,* 2014). This method is very helpful in the processes of selection. Combining genetic markers and phenotypic observation can increase the opportunity to evaluate plant characteristics.

Genetic markers have been useful for identifying plant genetic diversity. The identification is based on DNA polymorphism. The advantages of genetic analysis using molecular markers are: small amounts of plant samples are required, there are no environmental effects, and they are superior in evaluating diversity compared to other conventional techniques (i.e. based on morphology and biochemistry). Randomly amplified polymorphic DNA (RAPD) is one of the markers methods used to identify molecular polymorphism. This marker system has been used to analyze the diversity of rice (Rahman *et al*., 2007; Skaria and Muneer, 2011, Maulana *et al.,* 2014; Saker *et al.,* 2005). Besides RAPD, simple sequence repeat (SSR) technique and amplified fragment length polymorphism (AFLP) have also been used to evaluate rice genetic diversity (Saker *et al*., 2005; Rathi and Sarma 2012). The result based on RAPD markers for 18 local rice varieties from Tana Toraja and Enrekang Indonesia showed a high level of diversity (Maulana *et al*., 2014). The result of genetic diversity analysis on 299 varieties of *indica* inbred rice with (intron length polymorphism (ILP) and SSR markers indicated a low level of genetic diversity (Ming *et al*., 2015). The research in our study aimed to study genetic variation of upland local glutinous rice using morphology and RAPD markers, in order to identify potential parents to create a new best variety.

MATERIALS AND METHODS

Materials used for this experiment consisted of eight cultivars of local glutinous rice varieties from West Java, Banten and Bengkulu province (Table 1). The experiment was carried out from June 2016 to February 2017 in the experimental fields and laboratory of agronomy for evaluation biotechnological products, Research Center for Biotechnology, Indonesian Institute of Sciences.

Table 1. Genetic materials used in the study.

Cultivars	Origin Province
Pisitan Bulu	Banten
Ketan Hitam 46	West Java
Ketan Mayang	Banten
Ketan Jalupang	Banten
Ketan Hitam	West Java
Ketan Simpay	Banten
Ketan Hideung	Banten
Ketan Kelapa	Bengkulu

Analysis of agro-morphological characteristic in the field

The study did not use a replicated experimental design however each cultivar was planted randomly. All cultivars were planted on dry land (i.e. upland). The replication was performed by individual diversity of each cultivar; five plants of each cultivar were used as plant sample for agro-morphological trait characterization. Five plants were randomly taken as samples, which represent 30% of the population. From each sample, three random panicles were used for observing seed and panicle characteristics.

Seeds from each cultivar were planted in a 1 m^2 plot with plant spacing 25 x 25 cm. The seeds were planted by the drilling system with 3-5 seed/hole. Fertilizer application was using 150 kg/ha urea, 75 kg/ha TSP, and 50 kg/ha KCl. The observation was done in vegetative, generative, and post-harvest stage. Morphological and agronomic characters were observed referring to the Guidelines of Rice Characterization and Evaluation

System, National Commission of Germplasm, Agency for Agricultural Research and Development 2003. Coefficient of varians (CV) measured by the formula:

$$CV = \frac{S}{X}\, x\ 100\%$$

Where CV: Coefficient of variants, S: standard deviation, X: means of sample. The diversity is said to be high if the CV value is more than 50%.

Molecular marker analysis

DNA Extraction

Ten seeds from each cultivar were germinated on Yoshida solution for 14 days. The seeds were used for DNA analysis. DNA extraction was carried out by following CTAB method reported by Doyle and Doyle (1990) by modification. The leaves were ground in 2 ml tube with liquid nitrogen. CTAB buffer (2% CTAB, 100mM Tris-HCl pH 8.0, 1.4M NaCl, 20mM EDTA, 4% polyvinylpyrrolidone, and 0.5% mercaptoethanol; 500µl) was added to the leaf powder, which was then homogenized. The samples were incubated at 65ºC for 1 hour, and every 10 minutes the solution was homogenized. A volume of 500µl of chloroform and isoamyl alcohol (24:1) was then added into sample solution and homogenized. The samples were centrifuged at 12,000 rpm for 10 minutes. The supernatant was then transferred into a new tube, and 7.5M ammonium Acetate (CH_3COONa) was added until final concentration 0.6M. Solution added with 2/3 volume isopropanol and incubated overnight. The sample was centrifuged at 12,000 rpm for 10 minutes and the supernatant was discarded. The pellet was washed using 500 µl of 70% ethanol, and centrifuged at 12,000 rpm for 10 minutes. The supernatant was discarded and this was repeated by washing twice. The pellet was dried at room temperature and dissolved in 10-20 µl of Tris-EDTA buffer, and DNA was stored at -20 ºC.

RAPD analysis

In this study, the RAPD markers were used to evaludate genetic diversity because there is no information in glutinous rice genotypes. The RAPD technique is a fast and efficient method for assessing variation in genetic resources (Rabbani *et al*, 2008; Rahman *et al*, 2007; Mani *et al*, 2010). Information regarding diversity is important for plant breeding. If the results indicate a wide diversity, then the opportunities of obtaining superior characters in breeding populations is greater.

RAPD analysis was performed using sixteen random primers (Table 2). After this process, only 8 random primers showed clear polymorphic markers. PCR amplification was performed using KAPA 2G Fast HotStart PCR Kit with 2.5 µl (5x) Kapa G-Buffer A or M; 0.25 µl (10mM) dNTP mix; 0.5 (10 µM) Primer RAPD; 0.5 µl (100ng/µl) DNA of glutinous rice samples; 0.05 µl (5 u/µl) KAPA 2G Fast HotStart DNA Polymerase. PCR adjustment with pre-denaturation at 95 ºC, 3 minutes then 40 cycles of amplification (denaturation at 95 ºC, 15 seconds; annealing at 38-40 ºC, 15 seconds, elongation 72 ºC, 15 seconds) and a final elongation step

Table 2. Primers used.

Primers	DNA sequence
OPA 13*	5'-CAG CAC CCA C-3'
OPC 01	5'-TTC GAG CCA T-3'
OPAM 01	5'-TCA CGT ACG G-3'
OPAM 03	5'-CTT CCC TGT G-3'
OPB 14	5'-TCC GCT CTG G-3'
OPB 01*	5'-GTT TCG CTC C-3'
OPD 08*	5'-GGG TAA CGC C-3'
OPF 07*	5'-CCG ATA TCC C-3'
OPG 13*	5'-CTC TCC GCC A-3'
OPG 18*	5'-GGC TCA TGT G-3'
OPH 03*	5'-AGA CGT CCA C-3'
OPS 19	5'-GAG TCA GCA G-3'
OPW 05*	5'-GGC GGA TAA G-3'
OPW 16	5'-CAG CCT ACC A-3'
OPY 08	5'-AGG CAG AGC A-3'
OPZ 03	5'-CAG CAC CGC A-3'

* use for genetic analysis

(72 ºC, 10 minutes). Visualization was performed using 1.5% agarose with 0.01% Sybr safe DNA gel stain. The electrophoresis was at 45 volts for 2 hours in 1x TBE buffer solution. The result of electrophoresis was visualized and saved using gel documentation unit.

Data analysis

DNA fragments were converted into binary data. Value of 1 indicated the presence of a DNA marker whereas a value of 0 means there is no DNA band. The data was analyzed using Numeral Taxonomy and Multivariate Analysis System (NTSys) version 2.02. The results a generated a dendrogram which was based on genetic similarity.

Disease Inventory on glutinous rice plant

An inventory of plant disease was done directly to all disease symptoms that arise when planting (infection naturally). The observation was performed on the 118th day after planting (DAP) when maximum symptoms were observed. Scoring of plant damage referred to the Standard Evaluation System for Rice, IRRI (2013) (Table 3).

Disease severity (DS) was definied as the severity of disease damage and yield loss observed on a plant, calculated using the following formula (Gashaw *et al.,* 2014):

$$\mathrm{DS} = \frac{\sum(\mathrm{n\ x\ v})}{\mathrm{Nx\ V}}\ \mathrm{x}\ 100\%$$

Where, (DS) = disease severity; (n) = number of plant in each category; (v) =Numerical values of symptom category; (N) = Total Number of plants; (V) = maximum value of symptom category.

Disease Incidence (DI) as the presence or absence of disease, calculated using the following formula (Gashaw *et al.,* 2014):

$$\mathrm{DI} = \frac{\text{Number of infected plant units}}{\text{Total number of units assessed}}\ \mathrm{x}\ 100\%$$

Plant resistance to brown spot (BS) was categorized as follows

Table 3. The level of plant damage based on *Standard Evaluation System for Rice*, IRRI (2013).

Scale	ShB	BS	NBLS	BB	Blast
0	No infection observed	No disease observed	No disease observed	No disease observed	No lesions observed
1	Lesions limited to lower 20% of the plant height	Less than 1%	Less than 1%	-5%	Small brown specks of pin-point size or larger brown specks without sporulation center
2	-	1-3%	-	-	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves.
3	20-30%	4-5%	1-5%	6-12%	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves
4	-	6-10%	-	-	Typical susceptible blast lesions2 3 mm or longer, infecting less than 4% of the leaf area
5	31-45%	11-15%	6-25%	3-25%	Typical blast lesions infecting 4-10% of the leaf area
6	-	16-25%	-	-	Typical blast lesions infection 11-25% of the leaf area
7	46-65%	26-50%	26-50%	26-50%	Typical blast lesions infection 26-50% of the leaf area
8	-	51-75%	-	-	Typical blast lesions infection 51-75% of the leaf area and many leaves are dead
9	More than 65%	76-100%	51-100%	51-100%	More than 75% leaf area affected

ShB = Sheat Blight; BS= Brown Spot; NBLS=Narrow Brown Leaf Spot; BB =Bacterial Blight

(Djunaedy, 2009): Immune (0%); resistant (1-5%); moderately resistant (>5-10%); moderately susceptible (>10-25); susceptible (>25-50%); highly susceptible (>50%). For bacterial blight (BB), plant resistance can be categorized as follows (Adhikari and Mew, 1994): resistant (0-25%); moderately resistant (25.1-50%); susceptible (above 50%). According Puri *et al.* (2006), plant resistance to blast can be categorized as follows: immune (0%); resistant (1-15%); moderately resistant (15.1-30%); moderately susceptible (30.1-50%); susceptible (50.1-100%). For sheath blight (ShB) and narrow brown leaf spot (NBLS), Plant resistance were categorized based on existing damage in the field adjusted to Standard Evaluation System for Rice, IRRI (2013). Plant resistance to ShB was categorized as follows: immune (0%); resistant (lower 20%); moderately resistant (20-30%); moderately susceptible (31-45%); susceptible (46-65%); highly susceptible (above 65%). Plant resistance to NBLS was categorized as follows: Immune (0%); resistant (less than 1%); moderately resistant (1-5%); moderately susceptible (6-25%); susceptible 26-50%); highly susceptible (above 50%).

RESULTS

Agro-morphological characteristics

Performance data of glutinous rice is shown in Tables 4-8 and Figures 1-3. The variation of the 8 glutinous rice cultivars were tested based on the coefficient of variance value of quantitative characters. Among 10 variables observed, the highest variation was detected in number of the filled grain and the lowest is in 100 grains weight. This phenomenon indicated that in general, yield was not different. Table 4 shows that there are no differences at the quantitative leaf character of the glutinous plant, except cv. Ketan Mayang with purple color of hypocotyls and leaf midrib green color with purple lines. Generally, all cultivars had leaf 73.4 cm long and 2.7 cm wide), except cv. Ketan Kelapa and cv. Ketan Hitam-46 shorter (Table 5).

For harvest age characters, generally all cultivars have longer planting periods (Table 6), based on flowering data. This indicated that all cultivars had a longer time at vegetative phase and making the time for harvest longer. Based on the quantitative character of panicle (Table 7), Ketan Hideung is the highest yielding cultivar. Ketan Hideung has several features regarding agro-morphological characters such as: the longest panicle length, the greatest panicle weight, and the highest number of the productive tillers. Regarding panicle type characters, all cultivars had solid and moderate panicles. The range of numbers of empty grains was between 31-60%. Cv. Ketan Hideung had the lowest empty grains (31%), in contrast to cv. Ketan Hitam which had the highest (60%). This character can result in low yield of local glutinous rice varieties and needs improvement in the plant breeding program.

There was variation in grain morphology of each cultivar observed (Table 8). The weight of 100 grains ranged from 1.8 g (Ketan Kelapa) and the highest was Pisitan Bulu (3.1 g). Five cultivars had awns on the grain tip, while cultivars Ketan Hitam-46, Simpay and Kelapa were awnless. The cv. Ketan Jalupang had the longest awns (8.4 cm) compared to the others. Meanwhile, cv. Pisitan Bulu and cv. Ketan Hitam-46 had the lowest awns (0.4 cm). The presence of the awn on the tips of grains could protect the plant from bird pest. Based on Table 8, the data indicated that there was a correlation between color of grain tip and color of awn on the grain tip. All cultivars with awns had the same color for both characters, except Ketan Mayang.

Seed character results indicated that almost all cultivars tested had a slim and long seed, except Ketan Simpay and Ketan Kelapa (Table 9). Five of them had the aleuron color which was brown to black. The colored grain indicated high antioxidant content. There was a positive correlation between the content of amylose and antioxidant compounds (melatonin and phenolic). The seed length character of glutinous rice is different in each cultivar. There were two cultivars that were categorized as long (Pisitan Bulu and Ketan Hitam), while two other cultivars were categorized as short (Ketan Simpay and Ketan Kelapa) and the rest were medium.

Table 4. Coefficient of variation of local glutinous rice for some quantitative variables.

Quantitative trait	CV (%)
Leaf length	9.4
Leaf width	7.2
Plant high	8.4
Number of tillers	28.3
Number of productive tillers	27.8
Panicle length	6.0
Weight of panicles	23.4
Number of filled grains	36.5
Number of empty grains	29.3
Weight of 100 grains	2.2

CV=coefficient of Variants (variation said to be high if the CV value is more than 50%)

Table 5. Leaf characters of local glutinous rice.

Cultivars	Leaf surface	Flag leaf angle	Color of hypocotile	Color of node	Color of leaf strands	Color of leaf sheath	Shape of ligule	Leaf Length (cm)	Leaf Width (cm)
Pisitan Bulu	Rough	straighten	Green	Green	Black Green	Green	Acute-acuminate	72.3±6.4	2.9±0.1
Ketan Hitam 46	Rough	medium	Green	Green	Green	Green	2-cleft	38.1±9.5	0.9±0.1
Ketan Mayang	Rough	medium	Purple	Green	Green	Green with purple strip	Acute-acuminate	83.1±5.3	2.4±0.1
Ketan Jalupang	Rough	medium	Green	Green	Green	Green with White strip	Acute-acuminate	79.0±6.9	2.3±0.2
Ketan Hitam	Rough	medium	Green	Green	Green	Green with purple strip	Acute-acuminate	72.9±5.3	2.3±0.1
Ketan Simpay	Rough	medium	Green	Green	Black Green	Green	Acute-acuminate	63.3±5.1	2.0±0.3
Ketan Hideung	Rough	medium-drooping	Green	Green	Green	Green	Acute-acuminate	71.8±7.2	2.1±0.2
Ketan Kelapa	Rough	medium	Green	Green	Green	Green	Acute-acuminate	40.8±2.8	1.2±0

Table 6. Performance of local glutinous rice.

Cultivars	Clump shape	50% Days Flowering (DAP)	Plant Height (cm)	Number of Tillers	Number of Productive Tillers	Capability to Produce Tiller
Pisitan Bulu	erect-intermediate	90	199.9±27.7	8.2±2.5	8.6±1.8	A few
Ketan Hitam 46	Intermediate	78	116.4±10.6	19.4±9.5	15.6.2±6.4	A lot
Ketan Mayang	Intermediate	91	192.3±23.4	8.2±1.8	8.2±1.8	A few
Ketan Jalupang	Intermediate	98	211.4±11.6	9.6±1.7	8.0±2.0	Moderate
Ketan Hitam	Intermediate	91	180.4±20.5	12.6±1.7	10.2±2.5	Moderate
Ketan Simpay	Intermediate	82	149.2±8.4	7.6±3.4	7.4±3.5	A few
Ketan Hideung	Intermediate-descending	105	182.4±8.4	8.4±2.2	4.2±1.3	A few
Ketan Kelapa	Intermediate	81	115.2±3.8	25.8±5.5	21.0±3.2	A lot

DAP: day after planting

Table 7. Panicle characters of local glutinous rice.

Cultivars	Panicle Length (cm)	Panicle Weight (g)	Number of Filled Grain	Empty Grains (%)	Panicle Type
Pisitan Bulu	33.6±2.1	4.3±1.0	108.8±32.4	33.3	Between solid-moderate
Ketan Hitam 46	19.1±1.9	1.1±0.2	40.4±14.2	41.4	Between solid-moderate
Ketan Mayang	31.6±1.3	4.7±1.2	142.2±38.0	43.1	Solid
Ketan Jalupang	33.3±2.0	3.7±0.5	99.2±25.7	58.1	Solid
Ketan Hitam	34.2±1.7	2.1±0.5	58.4±18.8	60.2	Between solid-moderate
Ketan Simpay	26.3±1.5	4.1±1.2	143.0±75.9	53.2	Between solid-moderate
Ketan Hideung	43.5±1.7	6.5±1.3	159.8±63.2	31.2	Between solid-moderate
Ketan Kelapa	21.3±2.4	1.3±0.5	57.6±27.3	55	Moderate

Table 8. Grains characteristics of local glutinous rice.

Cultivars	Color of tip grain	Awn on tip grain	Color of Awn	Awn Length (cm)	Weight of 100 grains (g)
Pisitan Bulu	Yellow	Short, awn	Straw	0.4±0.1	3.1
Ketan Hitam 46	Brown	Non awn	-	-	2.1
Ketan Mayang	Yellow	Long, awn all panicle	Purple-back	6.5±0.4	2.9
Ketan Jalupang	Yellow	Long, awn all panicle	Straw	8.4±1.0	2.6
Ketan Hitam	Black	Long, awn all panicle	Black	0.4±0.2	2.8
Ketan Simpay	Straw	Non awn	-	-	2.1
Ketan Hideung	Straw	Long, awn all panicle	Straw	7.0±1.0	2.9
Ketan Kelapa	Yellow	Non awn	-	-	1.8

(–) not observed

Table 9. Grain characteristics of local glutinous cultivar rice.

Cultivar	Aleuron color	Length rice	Categories of rice length	Width rice	Rice Ratio	Rice category
Pisitan Bulu	Brown	6.93	Long	1.97	3.52	Slim
Ketan Hitam 46	Red-black	6.06	Middle	1.66	3.68	Slim
Ketan Mayang	Brown	6.38	Middle	1.75	3.65	slim
Ketan Jalupang	White	6.39	Middle	1.8	3.56	slim
Ketan Hitam	Red	7.11	Long	1.81	3.93	slim
Ketan Simpay	White	5.34	Short	1.69	3.18	slim
Ketan Hideung	Black	6.72	Middle	1.81	3.72	slim
Ketan Kelapa	White	5.37	Short	1.63	3.29	slim

Long category: very long (> 7.50 mm), long (6.61-7.50 mm), middle (5.51-6.60 mm), short (< 5.50 mm). Rice category base on ratio long: width: slim (> 3.0), middle (2.1-3.0), around grain shape (1.0-2.0) (Santika, 2010).

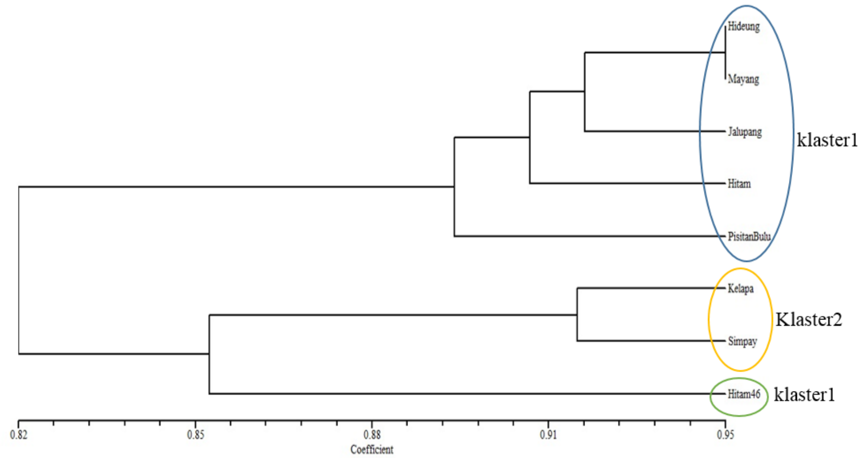

Figure 1. Dendrogram based on molecular analysis.

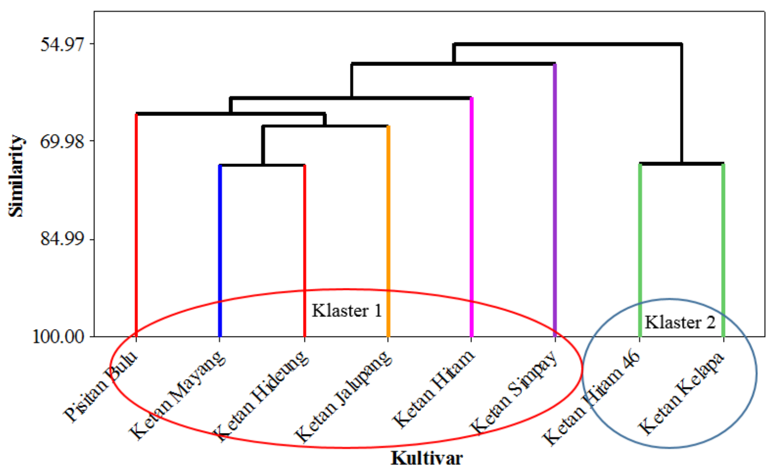

Figure 2. Dendrogram based on all quantitative characters.

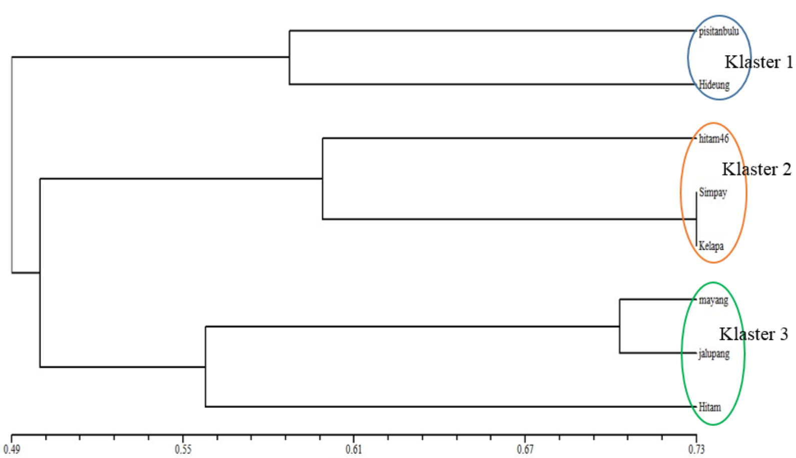

Figure 3. Dendrogram based on all qualitative characters.

Genetic diversity

Analysis of genetic characters was performed using RAPD. From sixteen primers, only eight primers produced polymorphism and clear markers. There were two groups based on molecular analysis at 85% similarity level (Figure 1). Genetic clustering reveals similarities or differences in plant characteristics. The determination of parental crosses should be based on different characters as far as possible, in order to obtain high diversity in offspring. High diversity will facilitate the process of plant selection. The result of cluster analysis based on quantitative and qualitative characters (Figures 1, 2 and 3) indicated that Ketan Kepala and Ketan Hitam-46 were in the same group. Figure 2 shows that two groups of were formed based on quantitative characters at 56.9% similarity level. Cultivars Ketan Hitam and Ketan Kelapa were in the same group and have the closest similarity. Based on plant character data, both cultivars have the lowest leaf length and width, short height, high tiller number, short panicles, and low panicle weight compared others. Figure 3 shows that three groups of plants were formed based on qualitative characters at 55% similarity level. Pisitan Bulu was similar to Ketan Hideung and was in the same group. Ketan Hitam-46, Ketan Simpay and Ketan Kelapa were in another group without awns. The last group members included Ketan Mayang, Ketan Jalupang and Ketan Hitam.

Disease inventory on glutinous rice plant

Disease results included five: sheath blight (ShB), brown spot (BS), blast, narrow brown leaf spot (NBLS), and bacterial blight (BB) (Table 10). In general, all cultivars were resistant to blast except cv. Ketan Hitam and Ketan Kelapa. Blast resistance is important for upland rice because blast is one of the major diseases in upland areas. All cultivars were resistant to ShB except cv. Ketan Hitam 46, Ketan Mayang and Ketan Hitam. Ketan Hitam and Ketan Hitam-46 resistant to BB. All cultivars tested were susceptible to BS expect Ketan Mayang. Cultivar Pisitan Bulu and Ketan Jalupang have potential to be a new varieties with ShB, blast, and NBLS resistance. Pisitan Bulu and Ketan Jalupang which had the strongest resistance to the diseases compared to other cultivars, but they were susceptible to BS and BB. The resistances of the disease of glutinous rice still need to further study to determine the most suitable varieties for specific regions and appropriate cultivation practices.

DISCUSSION

Diversity of eight local glutinous tested was low, except for number of the filled grains and 100 grain weight. This result is similar with another upland rice experiment which concluded that phenotypic and genotypic coefficient of variance (CV) was high for the number of the filled grains/panicle and yield/plant based on quantitative traits (Tuhina-Khatun, 2015). From the data, it can be concluded that almost all cultivars tested had similar plant height >120 cm (except Ketan Hitam-46 and Ketan Kelapa), low number of tillers (except Ketan Hitam-46 and Ketan Kelapa)

Table 10. Disease severity, disease incidence and resistance of local glutinous rice plant.

Cultivar	ShB			BS			NBLS			BB			Blas		
	DS (%)	DI (%)	PR	DS (%)	DI (%)	PR	DS (%)	DI (%)	PR	DS (%)	DI (%)	PR	DS (%)	DI (%)	PR
Pisitan Bulu	5.7	5.7	R	34.3	51.4	S	0.0	0.0	I	60.0	100.0	S	0.0	0.0	I
Ketan Hitam 46	71.4	100.0	HS	71.4	100.0	HS	12.0	2.9	MS	0.0	0.0	R	0.0	0.0	I
Ketan Mayang	25.7	42.9	MR	0.0	0.0	I	60.0	71.4	HS	82.9	42.9	S	0.0	0.0	I
Ketan Jalupang	11.4	11.4	R	34.3	91.4	S	0.0	0.0	I	54.3	100.0	S	0.0	0.0	I
Ketan Hitam	20.0	100.0	MR	62.9	28.6	HS	60.0	14.3	HS	8.6	14.3	R	33.3	5.7	MS
Ketan Simpay	14.3	69.0	R	48.6	93.1	S	68.0	17.2	HS	42.9	93.1	MR	0.0	0.0	I
Ketan Hideung	14.3	58.8	R	34.3	41.2	S	36.0	17.6	S	42.9	58.8	MR	0.0	0.0	I
Ketan Kelapa	14.3	60.0	R	45.7	100.0	S	0.0	0.0	I	37.1	100.0	MR	86.7	100.0	S

ShB: Sheath Blight; BS: Brown Spot; NBLS: Narrow Brown Leaf Spot; BB: Bacterial Blight; DS: Disease Severity; DI: Disease Incident; PR: Plant Resistance; I: Immune; R: Resistant; MR: Moderately Resistant; MS: Moderately Susceptible; S: Susceptible; HS: Highly Susceptible

and late maturity, but high in panicle number. Taller plants can fall down easily due to wind and rain or because of the stem unable to support the panicle weight during grain filling and ripening. Tall rice plants are susceptible to lodging because of the dry weight of straw and low stem strength with usually high panicle weight (Shinta *et al.,* 2014), and shorter plants with many tillers are more vulnerable to sheath blight disease (Nuryanto *et al*., 2014). The long panicles and high grain weight characters indicated high sink because it can accumulate assimilates from source (leaf and stem). The grain weight had a correlation with yield components in local purple glutinous rice (Khempet and Jongkaewwattana, 2012), and lower regression coefficient values indicated a higher assimilate efficiency for grain content (Tsukaguchi *et al.*, 2016). Experiments from China found that number of tillers, panicle length, the number of panicles, and yield component of glutinous rice were strongly influenced by different varieties (Miah *et al.,* 2008). Protein content, number of primary and secondary branches per panicle, number of productive tillers per clump, and the 1000 grain weight evaluated in 31 accessions of Bangladesh rice indicated some duplicates (Ahmed *et al*., 2016).

Seed characters of almost all cultivars have a long, slim and pigmented seed (five). Based on other experiments, 50 lines of upland glutinous rice have the lowest diversity including only five genotype with medium length (5.51-6.60 mm), and eight lines have a slim form of rice (ratio> 3.0), and all grains whitewash grains (Santika and Rozakurniati, 2010). The nutritional quality of purple rice genotypes (anthocyanin and antioxidative content) and intensity of pigmentation were strongly influenced by environmental factors (Rerkasem *et al*., 2015). This is particularly the bran pigment which contributes most to the phenolic level of the grain compared to the amylose content (Setyaningsih *et al*., 2015) and the total phenolic content was significant positively correlated with the panicle length of local purple glutinous (Khempet and Jongkaewwattana, 2012).

All cultivars had potential resistance to blast, except Ketan Hitam and Ketan Kelapa. Ketan Hitam is a highly susceptible cultivar causing a decrease in yield. Plant protection at an early growth stage is necessary to produce high yields. Resistance to disease can be developed by improving plant genetics (i.e. host plant resistance). Identification and breeding to incorporate specific resistance genes is important further work because of changes in virulence of the pathogen. The short-grain rice varieties with many tillers planted in lowland areas (0-200 m above sea level) showed more severe disease symptoms in all growing seasons compared to high-performance varieties, and had fewer seedlings (Nuryanto *et al*., 2014). The leaf blast and sheath blight simulations were developed based on yearly changes of mean temperature and relative humidity, increased frequency and intensity of climate extremes with greater climate variability; these results indicated reductions in crop yields and also altered dynamics of plant diseases and pests (Kim *et al*., 2015). Rice blast is a major disease in all the rice growing countries; it can cause 40% – 70% loss of rice grain (Challagulla *et al.*, 2015).

In this study, three dendograms were constructed based on genetic, qualitative and quantitative data, and all three of them showed similar groupings. Ketan Hitam-46 and Ketan Kelapa were always in the same group in all dendograms. This indicated that the both cultivars were similar, although their origin was different (Ketan Hitam-46 from West Java, Ketan Kelapa from Bengkulu Province). An experiment from Guangdong, China showed that genetic backgrounds due to regions and groupings contribute very little to the genetic diversity of 299 varieties of indica inbred rice (Ming *et al*., 2015). In another experiment, evaluation of 31 genotypes of Bangladesh rice accessions with the same name for morpho-phytochemical characters showed that genotypes in the same group did not originate from the same geographic location, and there was no relationship between genetic and geographic differences (Ahmed *et al*., 2016). The Southeast Asian origin for glutinous rice is consistent with Asian cultural practices (Olsen and Purugganan, 2002). The result of this research indicated that the use of RAPD markers combined with phenotypic data was useful for identifying genetic diversity of glutinous rice. Molecular markers such as SNPs (Susanto, 2015) and SSRs (Ahmed *et al*., 2016; Rathi and Sarma, 2012) can be used to distinguish genotype of rice based, but cannot determine their ancestors or derivatives.

The results of observations of qualitative and quantitative characters have been summarized in Table 10. Based on the data obtained, Pisitan Bulu is a cultivar with the most superior characters such us high production, good seed quality, and resistance to diseases. However, it needs to be improved for plant height, early flowering and the increasing number of tillers. According to Miah *et al*. (2008) number of tillers, panicle length, and a number of panicles and yield components on glutinous rice are strongly influenced by varieties. Cultivar Ketan Hitam-46 and Ketan Kelapa are candidate parents for crossing with short plant height, higher of a number of productive tillers, and early maturing, but low yield and susceptible to diseases. Pisitan Bulu and Ketan Hideung have a high yield potential compared to other cultivars. Overall from the data, Pisitan Bulu, Hideung, and Ketan Hitam-46 have high to be developed as new varieties of upland glutinous rice, or as parents for crossing in the future (Table 11).

Table 11. Summary of desirable characters for selection of parents for crossing.

Characters	Cultivars							
	Pisitan Bulu	Hitam46	Mayang	Jalupang	Hitam	Simpay	Hideung	Kelapa
Short plant		v						v
Erect of flag leaf	v							
Erect Plant	v							
Fast flowering		v				v		v
High tiller capability		v						v
High of productive tiller	v	v	v	V	v	v		v
Long panicle	v		v	V	v	v	v	
High number of filled grain	v						v	
Awning	v		v	V	v		v	
High of 100 grains weight	v		v				v	
Rice formed is slim	v	v	v	V	v	v	v	v
Grain length	v				v			
Colored aleuron	v	v	v	V	v		v	
Disease resistance								
Rice Sheath Blight	v							
Brown Leaf Spot			v					
Blas Blast	v	v	v	V		v	v	
Narrow Brown Leaf Spot	v			V				v
Rice Leaf Blight		v						
Total	13	8	8	7	6	5	7	6

CONCLUSION

Based on CV values, the variation within eight local glutinous rice cultivars tested was low. Pisitan Bulu and Ketan Hideung have potential to be used as parents for hybridization with the best yield component characters. However, they still need to be improved for some characters, such as: improve plant height to make it shorter, reduce maturity and more productive tillers. These weaknesses can be enhanced by Ketan Hitam-46 or Ketan Kelapa. Almost all cultivars tested have resistance to blast disease but Pisitan Bulu cultivar was the best resistance to various diseases.

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