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Identification of Genetic Relationship of Local Rice in East Java Based on gene *mat*K

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Abstract

Genetic diversity in living things is very important in the fulfillment of germplasm and conservation activities. East Java local rice is not so much cultivated farmers, so that the existence of local rice has begun to be replaced with new varieties and it is feared will happen genetic erosion of local rice. The purpose of this study to identify the genetic relationships of local rice and phylogenetic position of the rice contained in the Gene Bank. The method used to identify a molecular genetic using short pieces of DNA called DNA barcode. The results of this study indicate that the three local varieties of Berlian (Br), Genjah Harum (Gh) and Jawa (Jw) varieties can be identified by the matK gene barcode. The results of electrophoresis visualization showed that the DNA bands of the three samples were 900 bp. The results of DNA BLAST analysis show that the genetic relationships level with Oryza sativa and Oryza rufipogon is 100%. The results of the phylogenetic tree analysis showed that East Java's local rice was in one taxa with other rice and had a confidence level above 50 .

1. INTRODUCTION

Local rice is a type of wild rice that has long been cultivated by farmers in Indonesia. In the development of time, local rice has adapted from biotic or abiotic stresses in various parts of Indonesia, and can be used as a plasma nutfa that has various genes that control certain properties (Hairmansis *et al*., 2017). Local rice growth that is spread throughout Indonesia and cultivated by farmers has experienced adaptation to its environment. Adaptation that occurs in the local rice growing regions will form a local rice varieties are potentially like aroma rice, drought resistant, resistant to acid soil, and hold the flooded land (Sitaresmi, 2013). Local rice

productivity is very low when compared with the high-yielding rice varieties, but local rice has a very important role in the determination of superior varieties (Suhartini, 2010). Local rice diversity is a wealth of germplasm in Indonesia. The potential wealth of local rice germplasm in Indonesia is still not widely developed and identified phylogenetic (Nurhasanah, 2015).

Local rice research is an attempt to find and explore the genetic resources that could be used as a source of genetic wealth. Local rice genetic resources can be used to determine the phylogenetic position so that genetic information can be used as an assembly rice varieties that can produce a characteristic morphological and physiological properties better (Rabbani et al., 2008). The method used to identify local rice genetic that is molecular using short pieces of DNA called "DNA barcode" (Hebert *et al*. 2003). The method can be used even though the DNA of the organism is not in pure / intact form or that has been degraded (Hajibabaei *et al.* 2006).

Pieces of DNA that can be used as a specific marker gene *mat*K. *mat*K gene is a gene that is found in chloroplasts, have a more specific level of accuracy at the level of species, as well as used as an enzyme-coding sub unit maturase section K. The nucleotide region of the *mat*K gene has a length of around 1500 bp (Solis *et al*., 1998). The advantage of using *mat*K gene is that it can be used to distinguish a species, easily sequenced, easily amplified so as to produce a good nucleotide sequence (Hollingsworth *et al*., 2011). DNA barcoding research with *mat*K gene in plants angiosperms show *mat*K gene-sized fragment length 843 bp and thick and clearly visible in the visualization of UV-vis photo transilluminator (Kalangi., 2014).

2. MATERIALS AND METHODS

Tools and materials

The tools used in the study were hammer mortar, water batch, fortex, micropipette, centrifuge, electrophoresis, nanodrop, freezer and PCR machine. materials used in this study were agarose, distilled water, TBE, master mix for Intron PCR, universal matK primer and local rice leaves varieties of Berlian (Br), Genjah harum (Gh), and Jawa (Jw).

DNA Isolation

Stages of plant DNA isolation using the Illustra Nucleon Phytopure Genomic DNA Extraction Kits protocol. Total DNA was extracted from local rice leaves by 50 mg by adding lysis buffer solution. DNA bindings to filter sample DNA using the nucleon spin / collection tube. The DNA washing stage is used to purify the DNA with a washing buffer solution.

The last stage of DNA ellution by adding ellution buffer and the result of DNA isolation is stored in the freezer. The results of DNA isolation were further tested with nanodrop to determine the purity of DNA, the range of values of 1.8-2.0 showed pure DNA while below 1.8 indicates protein contamination and above 2.0 indicates RNA contamination.

Polymerase Chain Reation (PCR)

The PCR reaction in this study used PCR Mix introns with PCR composition, PCR Mix 10 µL, each Reverse and Forwad 1 µL primers, 7 µL SDW and 1 µL DNA template. the primer used is the matK_Forward gene primer TAATTTACGATCAATTCATTC, 1 µL primer matK_Reverse ACAAGAAAGTCGAAG TAT (Roy et al., 2015). The PCR reaction was pre-denatured at 94°C for 2 minutes, template DNA denaturation at 98°C for 30 seconds, anneling at 48°C for 40 seconds, then extention at 72°C for 1 minute, final extention at 72°C for 10 minutes and comprising of 40 cycles (Zodinpuii et al., 2013). DNA bands from PCR were electrophoresed using 1% agarose gel and visualized using UV-transilluminator. PCR products were subsequently sequenced at First Base Laboratories Sdn Bhd, Malaysia.

Data Analysis

DNA sequencing results in the form of chromatograms were analyzed using DNA baser software, Bioedit and FinchTV. DNA was analyzed at the software is further aligned by using the ClustalX to see the accuracy of the amplification of target genes by gene matK tested.

3. Results

Local rice samples taken from Banyuwangi that local rice varieties Berlian (Br) and Genjah harum (Gh), district local rice varieties Malang, Jawa (Jw). The nanodrop test was carried out to determine the quantity and purity of DNA, the results of nanodrop test on local varieties of Berlian (Br) varieties were 1.86, the sample was Genjah harum (Gh) 1.79 and samples of Jawa (Jw) varieties were 1.66. Results NanoDrop amplified by PCR machine and the result dielectrophoresis. The results of visualization of PCR products after electrophoresis showed the presence of DNA bands in the samples of Berlian (Br), Genjah harum (Gh) and Jawa (Jw) with 900 bp DNA fragment length.Electrophoresis of PCR result is shown in figure 1.

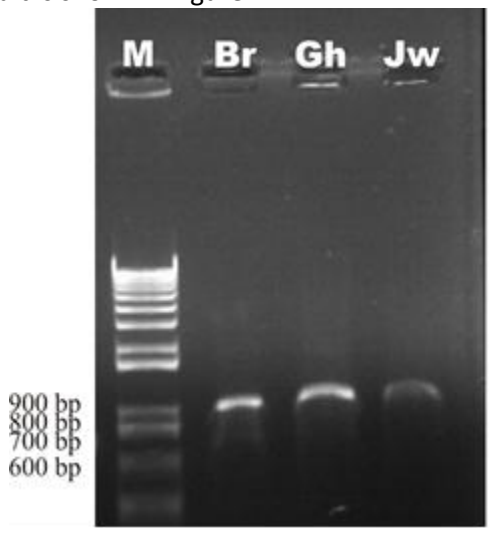


Figure 1. Electrophoresis of the East Java local rice matK gene band. M = 1kb marker plus DNA ladder; 1 = Berlian (Br); 2 = Genjah harum (Gh); 3 = Jawa (Jw)

Sequencing of local rice varieties Berlian, Genjah harum and Jawa have been successfully carried out with the nucleotide sequence length 910 bp. Furthermore, the nucleotide sequence was analyzed with BLAST.

The results of BLAST showed the max score of 1677 for varieties of Berlian (Br) and Genjah harum (Gh), while the Jawa variety (Jw) produced a max score of 1672. The query cover value of the varieties were Berlian (Br) and Genjah harum (Gh) (100%). , compare the query cover value on the Jawa variety (Jw) which is 99%. BLAST results can be observed in Figure 2 and Figure 3.

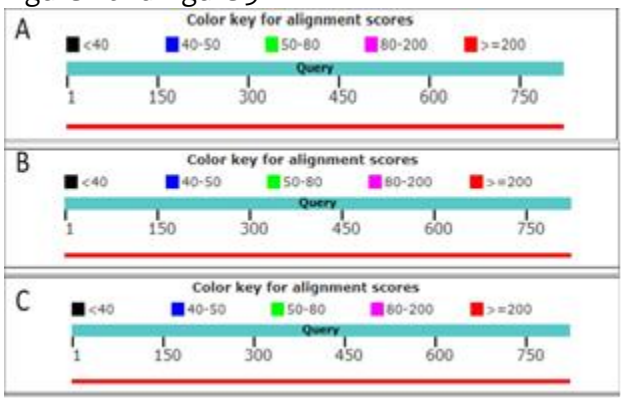


Figure 2. Results of analysis of local rice blast A. Berlian (Br), B. Genjah harum (Gh), C. Jawa (Jw) with comparator *Oryza sativa*

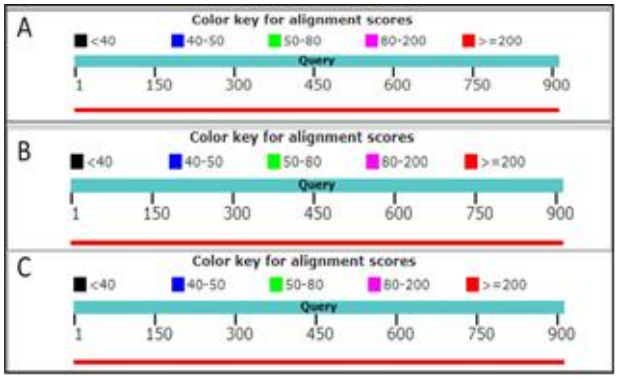


Figure 3. Results of analysis of local rice blast A. Berlian (Br), B. Genjah harum (Gh), C. Jawa (Jw) with comparator *Oryza rufipogon*

Analysis of nucleotide sequences using clustalX by aligning the three local rice samples. The juxtaposition of the three local

rice samples, the nucleotide sequence has much in common with local rice, is only found in the DNA sequence at the beginning of the Berlian (Br) and Genjah harum (Gh) sequences. The results of the alignment of DNA sequences are used for the reconstruction of phylogenetic trees by inserting local rice DNA sequences found in Gene Bank.

The results of the phylogenetic tree shows that local rice varieties of Berlian (Br), Genjah harum (Gh) and Jawa (Jw) one clade with rice samples contained in the gene bank that is Oryza sativa, Oryza rufipogon and Oryza barthii with confidence numbers 65 and samples proved different outgroup species to the local rice. Outgroup samples are Sorghum bicolor. Phylogenetic trees can be observed in Figure 4

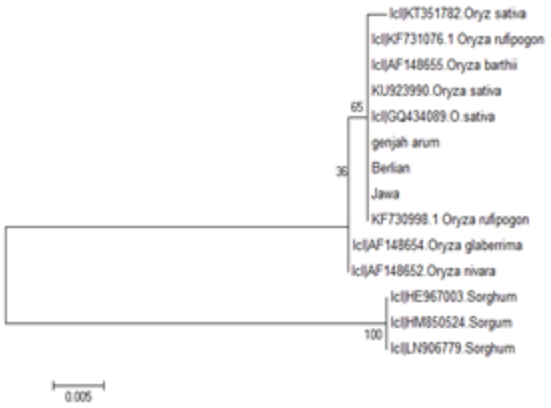
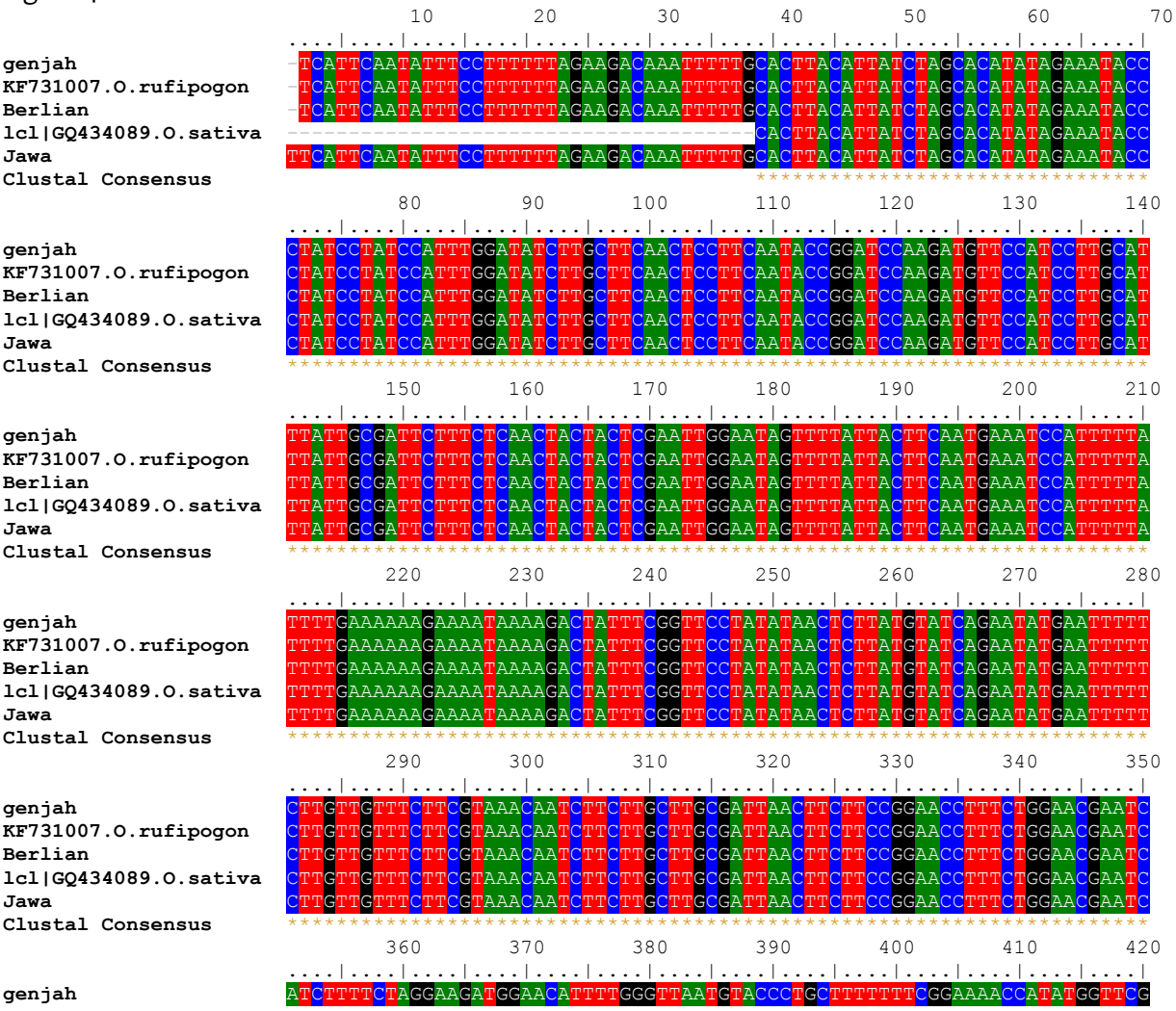


Figure 4. Phylogenetic trees from local rice samples of Berlian (Br), Genjah harum (Gh), dan Jawa (Jw).



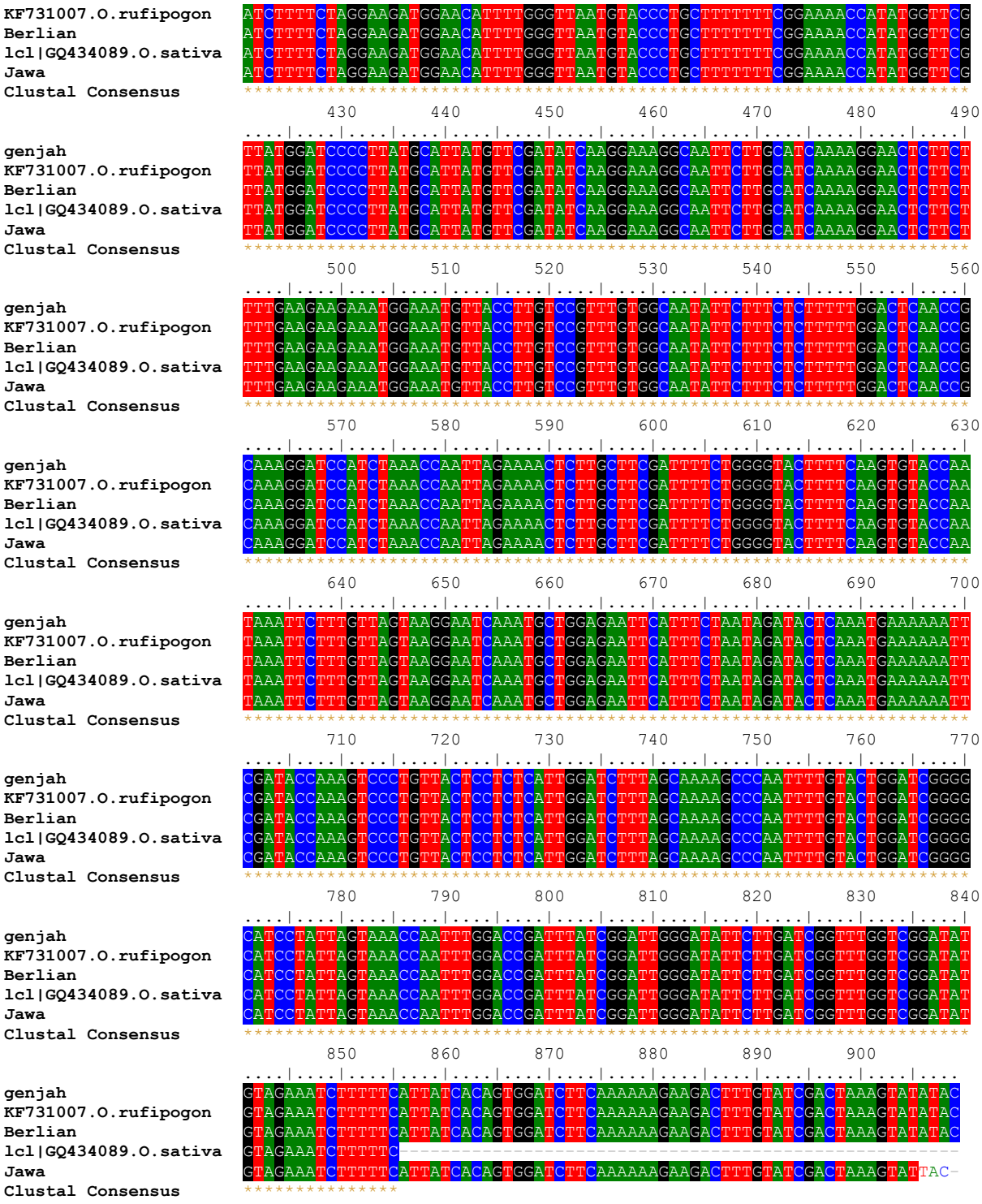


Figure 5. Alignment of East Java local rice *mat*K gene sequences with Oryza sativa (GQ434089) and Oryza rufipogon (KF731007).

The BLAST analysis result served as the basis in mining reference sequences from GenBank. In this research BR, SJ and JW were aligned with *Oryza sativa* Indica and *Oryza sativa* Japonica from GeneBank. This alignment was used as a basis to calculate the genetic distance of each sequence. The data from genetic distance was then used as a ground for phylogenetic reconstruction using the Neighbor-Joining method. According to neighbor-joining (NJ) tree reconstruction, BR, SJ, and JW formed a distinct cluster apart from *Oryza sativa* Indica and *Oryza sativa* Japonica. Within the local varieties cluster (BR, SJ, and JW), BR and SJ resided on the same clade despite some genetic divergences on BR side; both of them diverged from JW. All the three local varieties in this research split from the reference *Oryza sativa* Indica and *Oryza sativa* Japonica to form a different cluster, implying the genetic divergence of East Javan varieties (Fig. 5).

Based on the pairwise genetic distance values, all samples belonged to the same sub-species as all samples showed a low level of genetic divergence. However, as the genetic distance numbers on each sample compared to the reference sequences were all more than 0.000, these values indicated genetic variations or genetic divergences from that of Oryza sativa Indica and Oryza sativa Japonica (Table 2)Figure 4. B. cereus antagonist treatment against C. capsici; A is the appearance of the surface of the cup and the appearance of the cup. The red arrow indicates C. capsici mycelia and green arrows show B. cereus colonies.

4. DISCUSSION

The results of DNA isolation matK genes show DNA fragment thickness and clearly visible on elektrogram. At each sample showed DNA fragments of different thicknesses. In the Java variety sample (Jw) the thickness of DNA fragments is very thin. This can be caused by differences in extracted DNA concentration used during PCR (Sambrook & Russell, 2001).

BLAST analysis in local rice samples has a high max score. The max score is a value that shows similarity or identical sample nucleotide base pairs with comparison species (Nugraha et al. 2014). The higher the value, the higher the homologous level of the nucleotide sequence (Altschul et al., 1990). The max score that is owned by local rice has been confirmed to have similar nucleotides with comparative rice found in Gene Bank.

The nucleotide sequence is said to be identical if the query cover value is 40% (Daniels et al. 2013). The query cover value of the local rice sample is 100%. In the BLAST site, East Java local rice samples were also not found, indicating that blasting in local rice varieties Berlian (Br), Genjah Harum (Gh) and Jawa varieties (Jw) had never been carried out and this research was the first to be carried out.

Reconstruction of phylogenetic trees by placing a species other than rice ie wheat as outgroup. Outgroup addition is used to see and obtain convincing information from differences in local rice sample sequences ((Ward et al., 2008). Phylogenetic trees using the Neighbor Joining method can determine the proximity and genetic relationship distance of local rice samples with rice samples contained in the Gene Bank. , by determining the value of the boostrap (Ward et al., 2008). To evaluate the reconstruction of phylogeneous trees using bootstrap 1000 times to analyze the maximum parsimony and distance tree (Tjong, 2010).

5. Conclusion

The genetic relationship of local rice in East Java has been obtained by the length of the 900 bp DNA band sequence based on the matK gene. The results of the genetic relationship analysis show that the three local rice samples have the closest relatives to Oryza sativa, and Oryza rufipogon with a 100%

similarity level and had a confidence level above 50.

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