

**VARIASI DAN KEKERABATAN GENETIK KULTIVAR PADI LOKAL  
(*Oryza sativa* L.) LAHAN RAWA PASANG SURUT KALIMANTAN  
SELATAN BERDASARKAN PENANDA MORFOLOGIS,  
ULTRASTRUKTUR DAN MOLEKULAR**

Oleh :

**Dindin Hidayatul Mursyidin  
(NIM. 14/373996/SBI/125)**

**INTISARI**

Lahan rawa pasang surut Kalimantan Selatan memiliki beragam kultivar padi (*Oryza sativa* L.) lokal. Beberapa kultivar menunjukkan toleransi tinggi terhadap keasaman, salinitas, keracunan logam berat dan patogen. Namun demikian, sebagian besar kultivar tersebut belum dikaji secara mendalam untuk mendukung program pemuliaan tanaman pangan. Penelitian ini bertujuan untuk menentukan variasi genetik dan menganalisis hubungan kekerabatan genetik (fenetik dan filogenetik) kultivar padi lokal lahan rawa pasang surut Kalimantan Selatan berdasarkan penanda morfologis, ultrastruktur dan molekular.

Sebanyak 27 sampel padi lokal, terdiri atas 25 kultivar padi lokal lahan rawa pasang surut Kalimantan Selatan dan 2 kultivar pembanding (yaitu ‘Sardani’ dari lahan pasang surut Sumatera Selatan dan ‘Ciherang’ sebagai salah satu kultivar unggul nasional), telah dikarakterisasi secara morfologis menggunakan buku panduan *rice descriptor* (*Biodiversity International*). Sembilan dari 27 kultivar tersebut kemudian dikarakterisasi secara ultrastruktur menggunakan metode *Scanning Electron Microscopy* (SEM). Organ tanaman yang diamati dalam kegiatan ini adalah lidah daun (*ligule*) dan polen masing-masing kultivar. Dalam karakterisasi molekular, digunakan 10 sampel kultivar padi lokal sebagai wakil kluster hasil analisis morfologis. Dalam kegiatan ini, sampel DNA terlebih dahulu diisolasi dari daun tanaman padi berumur empat minggu menggunakan protokol kit ekstraksi DNA (Geneaid, UK). Sampel DNA kemudian diamplifikasi menggunakan metode PCR dengan primer *trnL-F* (cpDNA) dan IGS (rDNA), dipurifikasi dan setelah itu di-*sequencing* oleh 1<sup>st</sup> BASE Company, Singapura.

Analisis data dimulai dengan melakukan skoring dan standarisasi terhadap penanda morfologis dan ultrastruktur yang didapatkan. Setelah itu, variasi genetik masing-masing penanda ditentukan berdasarkan metode indeks Shannon-Weaver. Rekonstruksi kekerabatan fenetik kedua penanda tersebut dilakukan menggunakan metode UPGMA (*Unweighted Pair-Group Method with Arithmetic Average*) dan PCA (*Principal Component Analysis*) dengan bantuan *software* MVSP versi 3.1. Sementara itu, analisis data molekular dimulai dengan penggabungan sekuen DNA hasil *sequencing* menggunakan *software* Clustal X versi 2. Sekuen-sekuen DNA tersebut kemudian di-*alignment* menggunakan metode *Kimura-2 Parameters* dengan bantuan *software* MEGA7. Analisis variasi genetik penanda molekular dilakukan dengan metode indeks variasi nukleotida ( $\pi$ ), sedangkan rekonstruksi filogenetiknya menggunakan metode *maximum likelihood*, juga dengan bantuan *software* MEGA7.

Hasil penelitian menunjukkan bahwa padi lokal lahan rawa pasang surut memiliki variasi genetik rendah berdasarkan penanda morfologis, ditunjukkan dengan rerata indeks Shannon-Weaver sebesar 0,40. Namun demikian, percabangan malai sekunder dan distribusi bulu pada gabah merupakan dua penanda morfologis yang memiliki variasi genetik tinggi, masing-masing dengan indeks sebesar 0,93 dan 0,85. Berdasarkan analisis ultrastruktur, padi lokal juga menunjukkan variasi genetik rendah, dengan rerata indeks sebesar 0,43. Namun demikian, ukuran polen merupakan satu-satunya penanda ultrastruktur yang menunjukkan variasi genetik tinggi, dengan indeks sebesar 0,96. Sementara itu, hasil karakterisasi molekular menunjukkan bahwa padi lokal memiliki variasi genetik tinggi, ditunjukkan dengan rerata nilai variasi nukleotida sebesar 0,60. Variasi genetik tertinggi dalam penanda molekular ditunjukkan oleh penanda IGS (0,68). Hasil rekonstruksi fenetik menggunakan penanda morfologis dan ultrastruktur, mengungkapkan bahwa padi lokal mengelompok menjadi tiga kluster, dengan rentang similaritas masing-masing antara 54,6-91,0% dan 51,9-95,0%. Berdasarkan gabungan penanda molekular *trnL-F* dan IGS, padi lokal memisah dan mengelompok ke dalam tiga klad (cabang filogeni) utama, dengan rentang similaritas 88,4-98,2%.

Berdasarkan hasil ini dapat disimpulkan bahwa variasi genetik padi lokal berdasarkan penanda morfologis lebih rendah dibandingkan penanda ultrastruktur, sedangkan penanda ultrastruktur lebih rendah dibandingkan penanda molekular. Disamping itu, dapat disimpulkan pula bahwa berdasarkan penanda morfologis dan ultrastruktur, padi lokal memiliki hubungan kekerabatan genetik yang lebih jauh dibandingkan penanda molekular. Oleh karena itu, penanda molekular merupakan penanda ideal yang dapat diaplikasikan untuk menentukan tingkat variasi genetik dan merekonstruksi hubungan kekerabatan filogenetik padi lokal lahan rawa pasang surut, serta meningkatkan efisiensi program pemuliaan tanaman padi pada masa mendatang.

Katakunci : *Oryza sativa* L., variasi genetik, rawa pasang surut, ultrastruktur, filogenetik.

**GENETIC VARIATION AND RELATIONSHIP OF THE LOCAL RICE  
(*Oryza sativa* L.) CULTIVARS OF THE SOUTH KALIMANTAN'S  
TIDAL SWAMP AREAS BASED ON MORPHOLOGICAL,  
ULTRASTRUCTURAL AND MOLECULAR MARKERS**

By :

**Dindin Hidayatul Mursyidin  
(NIM. 14/373996/SBI/125)**

**ABSTRACT**

Tidal swamp areas of the South Kalimantan's Province, Indonesia have a large number of local rice cultivars (landraces). Several of this germplasm shows a good tolerance to acidity, salinity, heavy metals contamination, and pathogens. However, most of the local rice landraces are uncharacterized and underutilized, particularly in the conservation and rice breeding programs in Indonesia. The objectives of this study were to determine the genetic variation and analyze the phenetic and phylogenetic relationships of the tidal swamp rice cultivars of the South Kalimantan's Province based on morphological, ultrastructural, and molecular markers.

Twenty-seven cultivars of the local rice were used in this study, consisting of 25 cultivars of the tidal swamp rice from the South Kalimantan's Province, and two cultivars as a comparison (i.e. 'Sardani' from the tidal swamp areas of South Sumatera, and 'Ciherang' as one of the Indonesian superior cultivars). The study started with the characterization of the local rice using morphological markers, following a guide book of rice descriptor (Biodiversity International). The next activity was the characterization of local rice using SEM methods (ultrastructural analysis). Characterization performed using nine of 27 rice cultivars, where the ligule and the pollen of each samples were observed. The last activity was a molecular characterization, conducted using chloroplast DNA markers (*trnL-F*) and nuclear ribosomal DNA (IGS). In this activity, the DNAs isolated from the young leaves (four-week-old) of 10 rice samples using DNA extraction kit (Geneaid, UK). The DNAs then amplified using the PCR method, purified and sequenced by 1<sup>st</sup> BASE Company, Singapore.

Data analyses performed prior to the morphological and ultrastructural markers, by scoring and standardizing all the characters obtained. The genetic variation of both markers then determined by the Shannon-Weaver index method, with an assistance of MVSP version 3.1 software. The reconstruction of phenetic relationships of both markers was done using UPGMA (Unweighted Pair-Group Method with Arithmetic Average) and PCA (Principal Component Analysis) method, also with the assistance of MVSP versi 3.1. In addition, analysis of molecular data started by combining the DNA sequences, using a software of Clustal X version 2. The DNA sequences, then aligned using the Kimura-2 Parameter method by the assistance of MEGA7 software. The genetic variation analysis of this marker was performed by the index method of nucleotide variation

( $\pi$ ). The phylogenetic reconstruction of molecular marker was done by the maximum likelihood method, also with the assistance of MEGA7 software.

The results showed that the tidal swamp rice cultivars had a low genetic variation based on morphological marker, indicated by an average of Shannon-Weaver index of 0.40. However, secondary branching and awn distribution of the grains were two of morphological markers that show high genetic variation, with an index of 0.93 and 0.85, respectively. Based on ultrastructural analysis, the local rice also showed a low genetic variation, with an average index of 0.43. However, the area of pollen was the only ultrastructure marker that shows high genetic variation, with an index of 0.96. In addition, the results of molecular characterization revealed that this germplasm has a high genetic variation, indicated with a mean value of nucleotide variation of 0.60. The highest variation of this marker was pointed out by IGS sequences (0.68). The phenetic reconstruction of morphological and ultrastructural markers revealed that the local rice germplasm grouped into three clusters, with the ranges of similarity coefficients of 54.6-91.0% and 51.9-95.0%, respectively. In this study, a combined sequence of molecular markers (*trnL-F* and IGS) were able to produce phylogenetic trees and separate the germplasm into three clades, with a range of similarity coefficient of 88.4-98.2%.

Based on these results, it could be concluded that the genetic variation of the local rice was lower based on the morphological marker than the ultrastructure, whereas the ultrastructural marker was lower than the molecular. In addition, based on morphological and ultrastructural markers, the local rice germplasm shows a far genetic relationship. Contrarily, the molecular shows the close relationship. Thus, this marker, particularly the IGS, was an ideal for determining the extent of genetic variation and reconstruct the phylogenetic relationship of the tidal swamp rice cultivars. Hopefully, this marker may apply for improving the rice breeding program's efficiency in the future.

Keywords : *Oryza sativa* L., genetic variation, tidal swamps, ultrastructure, phylogenetic.