Stability Analysis of the Genetic Profile of Drought-Stressed Rice (*Oryza sativa* L.)

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Abstract. Global climate change might cause a region to have a prolonged dry or rainy season. A long dry season can create drought stress in rice plants, resulting in Reactive Oxygen Species (ROS) compounds in cells and causing damage to plants, one of which is damage to DNA. The purpose of this study is to examine the stability of rice's genetic profile in order to assess the effect of drought stress treatment on rice DNA itself. Previous research classified three rice varieties into three groups based on their drought stress resistance: Harum (tolerant), Situbagendit (moderate), and Rosna (sensitive). These three rice plants were germinated and then treated with drought stress using PEG. Rice roots before and after being treated with drought stress were collected and the DNA was extracted. Genetic profile stability analysis was carried out by RAPD PCR using 10 types of primers. The DNA band patterns in rice samples before and after drought stress were differed, according to the electropherograms of ten RAPD primers and Jaccard's similarity index. This suggests that drought stress may disrupt the genetic stability of the three rice varieties studied.

1 Introduction

Paddy (*Oryza sativa*) is a plant that has high economic value because almost half of the world's population uses rice as their main food ingredient. Indonesia is the third largest rice-consuming country in the world after China and India [1]. Global climate change can cause an area to experience a prolonged dry season or rainy season. Prolonged dry seasons can cause drought stress in rice plants which results in a decrease in rice yield and quality. The decline in rice production due to this drought can reach 58%. This drought condition will cause changes in the anatomy and morphology of rice. In general, rice grown in drought-stress areas will be smaller in size compared to rice grown in normal areas [2].

The mechanisms of tolerance to drought are well known. In the early stages, dryness causes reduced stomata opening to reduce water loss under excessive light conditions. This event resulted in a decrease in CO_2 concentration intracellularly, so that plants experience an over-reduction in photosynthetic electron transfer [3]. This causes the rise of Reactive Oxygen Species (ROS), which starts with electron binding to photosynthetic electron transport by oxygen. The following mechanism generates numerous ROS molecules such as

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superoxide anion radical, singlet oxygen, hydroperoxyl radical, hydroxyl radicals, and free nitrogen radicals [4,5]. Plants can be harmed by these ROS molecules [6]. At high quantities, ROS can affect physiological functions by causing cell damage to DNA, proteins, lipids, and other macromolecules [7]. If this is allowed to continue, the plant will eventually die [8, 9].

DNA stability and integrity are critical because DNA stores genetic information in every living cell. The ROS molecules created have the potential to harm DNA. Mutations will occur if DNA damage is not repaired [10]. DNA mutations can change a protein's amino acid sequence, which is crucial for responding to drought stress. Previous research has selected several rice varieties cultivated in West Sumatra. Selected rice varieties are grouped based on their level of resistance to drought conditions, namely tolerant rice (Harum and Baroto varieties), moderate rice (Situbagendit and Randam Kaus varieties), and sensitive rice (Keriting, Batang Palo, Kuning rendah, Indragiri, and Rosna putih varieties) [11]. However, it is not yet known whether drought stress can disrupt the stability and integrity of DNA in rice. Therefore, in order to establish the effect of drought stress treatment on rice DNA, the stability of the genetic profile of rice must be examined. The purpose of this study is to assess the effect of drought stress on the genetic stability of three groups of rice based on their level of drought resistance.

2 Materials and methods

This research was a qualitative descriptive study using three rice groups based on their drought stress resistance: Harum (tolerant), Situbagendit (moderate), and Rosna (sensitive).

2.1 Rice germination

Each type of rice is germinated in a germination container. Germination was carried out for 3 days using stencil paper as a medium in a dark room and at room temperature. Then the 3-day-old sprouts were germinated for five days in a box containing Yoshida's nutrient culture solution which was given a basket as a barrier between the roots and the shoot. The rice is placed in the basket in an upright position so that the roots can come into direct contact with the solution. Each root tip was cut as samples of drought stress pre-treatment and stored in DNA/RNA shield solution in a -20°C freezer until processed.

2.2 Treatment of drought stress with PEG

Seedlings of each variety that had uniform growth were transferred into boxes containing 20% PEG-6000 in 500 ml of Yoshida's solution to provide drought-stress conditions for five days. Then the root tips were cut as samples of drought stress post-treatment and stored in DNA/RNA shield solution in a -20°C freezer until processed.

2.3 DNA extraction

DNA was extracted from root tips (sample weight 50 mg) using Genezol according to manufacturing procedures. The results of DNA extraction from rice root tip samples were measured for DNA quality and quantity using a nano-spectrophotometer. The A260/A280 ratio of pure DNA is in the range of 1.8-2.0. The DNA concentration was adjusted to 100 ng/µl for RAPD PCR using TE buffer solution pH 8 or nuclease-free water to obtain a consistent DNA band profile in RAPD result.

2.4 Random amplified polymorphic DNA (RAPD) PCR

The RAPD PCR reaction used 10 commercially available primers, namely OPA-02, OPA-04, OPB-12, OPC-15, OPE-12, OPE-14, OPE-15, OPJ-20, OPM-09, and OPN-15. To achieve a consistent reaction between the pre-and post-treatment groups, the PCR reaction components (except for the DNA template) were mixed in a cocktail for all samples. The components per PCR tube consist of: 5 µl of 2x i-Taq PCR Master Mix Solution (Intron), 0.4 µl of 10 µM RAPD primer, 3.6 µl of nuclease-free water, and 1 µl of 100 ng/µl DNA template.

In this study, touchdown PCR was used to increase the specificity of the primers. The amplification profile was as follows: initial denaturation for 3 minutes at 94°C, then followed by two stages of the PCR cycle. The first stage consists of 10 cycles of denaturation at 94°C for 1 minute, annealing for 45 seconds at 42°C with a decrement of 0.5°C each cycle until it reaches 36°C, and elongation at 72°C for 2 minutes. The second stage consists of 30 cycles of denaturation at 94°C for 1 minute, annealing for 45 seconds at 36°C, and elongation at 72°C for 2 minutes. Then this amplification cycle will end with a final elongation at 72°C for 5 minutes. The PCR product was electrophoresed on a 1.5% agarose gel using a voltage of 100V for 30 minutes, and stained with GelRed (Biotium) then observed under UV light using Gel Doc [12].

2.5 Data analysis

Scoring was carried out based on the presence or absence of DNA bands in each sample. The score was 1 for a band that was present and a score of 0 for a DNA band that was absent. DNA bands with the same migration rate were assumed to be one homologous locus. Then, this data was analysed using Jaccard's similarity index by the PAST 4.08 programme.

3 Results and discussion

Ten RAPD markers were used for rice DNA profiling of drought-stress pre- and post-treatment. The electropherogram of each primer is presented in Fig. 1. The electropherogram of each primer revealed that the DNA band patterns generated differed between pre-and post-drought stress. For instance, the Harum variety (V1) RAPD PCR product using primer OPA-02 revealed changes in DNA band patterns between pre (V1A) and post-stress (V1B). V1B has two extra DNA bands between the 250 and 500 bp ladders, whereas V1A did not have two bands of this size. The Situbagendit variety experienced the similar phenomenon, as evidenced in the RAPD PCR primer OPN-15 result, in that there were 9 DNA bands before drought stress (V2A), but only 4 DNA bands after drought stress (V2B). The same result was seen in PCR products using other primers, such as the OPE-12 primer, where there were clearly obvious changes in DNA banding patterns between the Rosna varieties before (V3A) and after (V3B) drought stress treatment. In V3A, there are 14 DNA bands, whereas in V3B, there are just four. Other studies that used RAPD found that the DNA band pattern pre- and post-treatment of microradiation exposure to bacterial culture differed [13].

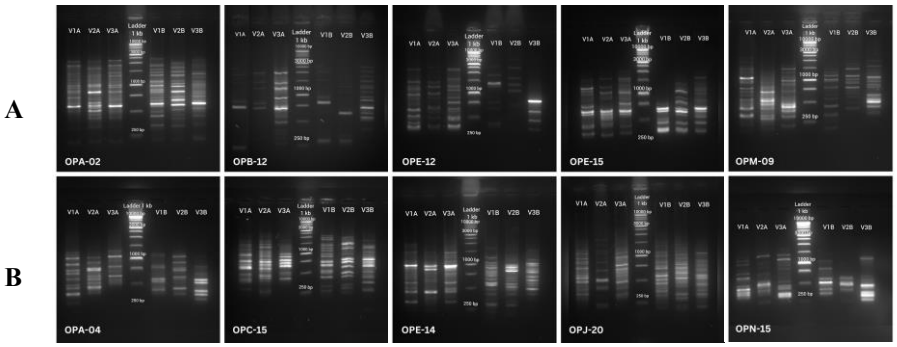
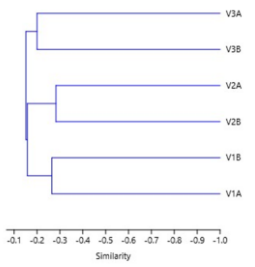


Fig. 1. RAPD electropherogram of drought-stressed rice DNA pre-treatment (A) and post-treatment (B). V1 = Harum (tolerant group); V2 = Situbagendit (moderate group); V3 = Rosna (sensitive group).

Jaccard's similarity index was used to compute similarity for RAPD data between pre- and post-treatment groups, and similarity calculations were analysed using the UPGMA algorithm. The resulting cluster was represented as a dendrogram (Fig. 2). The dendrogram revealed that each variety was clustered into three different branches. The upper, middle, and lower branches were the Rosna, Situbagendit, and Harum variety, respectively, which consist of pre-and post-drought stress treatment of each variety. These findings suggest that drought stress treatment alters the genetic profile of rice, but that each variety of pre-and post-treatment remains in the same cluster.



a

	V1A	V1B	V2A	V2B	V3A	V3B
V1A	1					
V1B	0,264516	1				
V2A	0,156627	0,116564	1			
V2B	0,176471	0,180124	0,282759	1		
V3A	0,180233	0,102857	0,18125	0,119318	1	0,2
V3B	0,131148	0,186747	0,142012	0,168605	0,2	1

b

Fig. 2. Jaccard's similarity dendrogram (left) and matrix (right) of drought-stressed rice DNA pre-treatment (A) and post-treatment (B). V1 = Harum (tolerant group); V2 = Situbagendit (moderate group); V3 = Rosna (sensitive group).

The Jaccard's similarity index is a common proximity measurement used to compute the similarity between two objects or two collections of data. The index, created by Paul Jaccard, ranges from 0 to 1. If two datasets have exactly the same members, their Jaccard Similarity Index is 1, and if they do not, their Jaccard Similarity Index is 0 [12]. This algorithm can be applied to assess the DNA stability of two sets of data [14], in this study between pre- and post-drought stress RAPD-based DNA profiles among three rice varieties. The algorithm will compare a number of the same DNA bands between pre-and post-drought stress to a number of all DNA bands that appeared. If the number of DNA bands is consistent between pre- and post-drought test then the Jaccard's similarity index is 1, thus the RAPD genetic profile of drought-stressed rice between pre-and post-treatment is similar or stable. However, if the number of DNA bands is inconsistent between the pre- and post-drought test then the

Jaccard's similarity value is close to 0, thus the RAPD genetic profile of drought-stressed rice is dissimilar or unstable. In response to drought stress, the most stable varieties are the Situbagendit variety, which was classed as moderate, while the most unstable is the Rosna variety, which was classified as sensitive. A great deal of research suggests that environmental variables such as heat, cold, drought, and infections can cause ROS production in plant cells. ROS induce irreversible cellular damage, including DNA damage, due to their strong oxidative qualities, which encourage morphological changes in plants that increase resistance [6]. Drought responses in rice crops are thought to be complicated, with a wide range of physiologic, biochemical, and molecular alterations [8].

4 Conclusion

According to the findings of this study, drought stress disrupts the stability of the rice genetic profile in all three varieties examined. The most stable is the Situbagendit variety, while the most unstable is the Rosna variety.

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