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Genetic Diversity Test of Various Javanese Local Sorghum Using RAPD Molecular Markers

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Abstract: Sorghum is one of the cereal crops that has good growth adaptation and production on dry land so that it has the potential to be developed in Indonesia. Besides being able to produce in dry areas, sorghum has a higher protein content compared to corn and cracked rice, but lower than wheat. Local sorghum used in this study included local Jember, local Demak, local Nganjuk, and local Majalengka. The level of genetic diversity of sorghum in Indonesia is still relatively low. Therefore, it is necessary to test the genetic diversity of sorghum with plant breeding. To obtain genetic diversity, this research conducted identification using RAPD molecular markers with 9 primers, namely OPA- 01, OPA-02, OPA-03, OPA-04, OPA-05, OPA- 06, OPA-11, OPA 17, and OPA 18. Based on the results of RAPD, the results of DNA bands were obtained which were continued with the NTSYSpc program to produce a kinship tree between local sorghum plants. From the results of the phylogenetic tree, it is known that the local sorghum of Majalengka and Lamongan have a parallel kinship, which means that the sorghum is genetically the same. Meanwhile, the genetic diversity of local sorghum in Jember and Demak is far compared to Lamongan and Majalengka. Nganjuk local sorghum has a genetic diversity line position in the middle (Jember, Demak) and (Majalengka, Nganjuk).

Keywords: Diversity, Genetics, Sorghum

INTRODUCTION

Indonesia is a country with a strategic location that has the potential to be independent in fulfilling national food needs. However, in reality, Indonesia still imports food from other countries to fulfil national needs such as Cambodia, Vietnam, and Myanmar [1]. Global phenomena such as changes in climate patterns are a challenge to national food security. Sorghum is one of the cereal crops that has good growth adaptation and production on dry land so that it has the potential to be developed in Indonesia. Besides being able to produce in dry areas, sorghum has a higher protein content compared to corn and cracked rice, but lower than wheat. In addition to consumption, sorghum plants are also useful as a source of bioenergy, food, and animal feed. Sorghum contains enough calcium, phosphorus, vitamin B1, and water. Sorghum has 332 kcal of energy and 73.0 g of carbohydrates per 100 grams [2].

Local sorghum veriatas are a type of sorghum owned by the community that already exists and is cultivated by local farmers for generations. This study used 5 types of local sorghum in Java, including Demak local sorghum, Jember local, Nganjuk local, Majalengka local and Lamnongan local. The selection of superior seeds for sorghum is still an obstacle in Indonesia. Based on this, many farmers are reluctant to plant sorghum. Compared to Asia or the world, the production of sorghum plants in Indonesia is still very small [3].

Based on its area, the sorghum plant in Indonesia is the largest in various regions, which is around 8,000 ha. [4] However, based on the level of genetic diversity in Indonesia, it is still relatively low. Therefore, it is

necessary to test the genetic diversity of sorghum with plant breeding. The thing that is mandatory in doing plant breeding is to carry out genetic diversity [5]. To find out the genetic diversity of some mutant local sorghum varities can certainly be done with the help of molecular markers [6] Selection of RAPD molecular markers in this study because RAPD is simpler and easier to do and produces many DNA bands. (Langga et al. 2012) Therefore, this study aims to determine the genetic diversity of various local sorghum in Java.

METHOD

This research was conducted in the Laboratory of UPA Waste Processing and Integrated Laboratory of Jember University on Tegalboto Street No 37, Krajan for 3 months from January to April 2024. To obtain genetic diversity, this research conducted identification using RAPD molecular markers with 9 primers, namely OPA- 01, OPA-02, OPA-03, OPA-04, OPA-05, OPA- 06, OPA-11, OPA 17, and OPA 18. Before conducting DNA isolation, first grow sorghum for 1 month, then isolate sorghum DNA using 0.5 gram leaf samples and using Tiangen Plant Genomic DNA kit. After that, it was spectrophotomrter 13,000 rpm for 15 minutes at 4 C. Then the DNA was checked for concentration using spectrophotometry and then PCR using Nextpro master mix. The next stage was agarose gel electrophoresis 25 for 40 minutes 80 volts. To observe the band of RAPD results placed on the documentation gel (UV Transilluminator) Then the number of bands counted on each primer. To find out the genetic similarity, it was calculated using the NTSYS phylogenetic tree so as to produce genetic closeness of various local sorrel of Java.

RESULTS AND DISCUSSION

The use of molecular markers is used to determine the traits resulting from mutation techniques. Molecular markers are methods used to determine genotype differences directly at the DNA level [7]. Plant breeding begins with improving the nature of a plant, followed by increasing its genetic diversity. The trait observed in conducting genetic diversity is DNA, because it has characters that are difficult to influence by the environment [8]. The diversity of genetic characters of each individual sorghum plant studied was produced during the amplification process that occurred during analysis using RAPD. Phenotypic differences of a particular trait do not always guarantee superiority and genetic differences [9]. The resulting polymorphism occurs because the primary nitrogenous bases are able to amplify DNA fragments properly. To obtain genetic diversity, this study identified using RAPD molecular markers with 9 primers namely OPA-01, OPA-02, OPA-03, OPA-04, OPA-05, OPA- 06, OPA-11, OPA 17, and OPA 18. The primers used are randomly sequenced primers and are not specific to certain genes so that the resulting bands are thought to represent new traits.

The description of local sorghum is addressed with the letters J, D, M and N. Local Jember (J), local Demak (D), local Nganjuk (N) and local Majalengka (M) and marker (m). The results of identification of DNA banding pattern of electrophoresis results from each primer in the figure below.

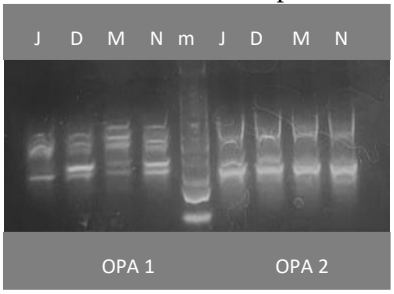

Figure 1. Visualisation of DNA bands from RAPD on primers OPA 1 and OPA 2.

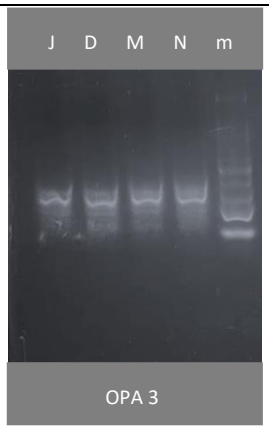

Figure 2. Visualisation of RAPD-derived DNA bands on primers OPA 3

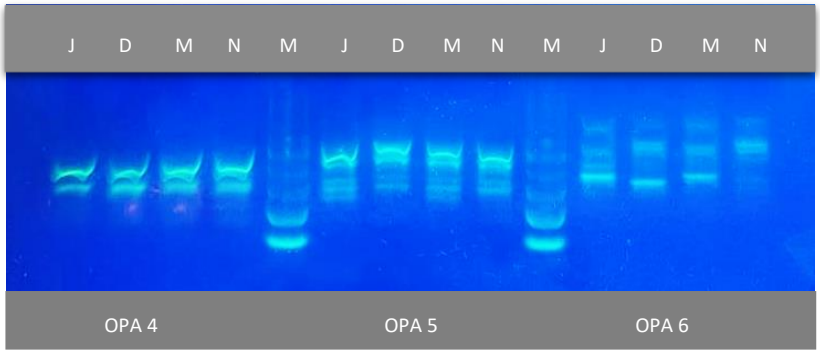

Figure 3. Visualisation of DNA bands from RAPD primers OPA 4, OPA 5 and OPA 6

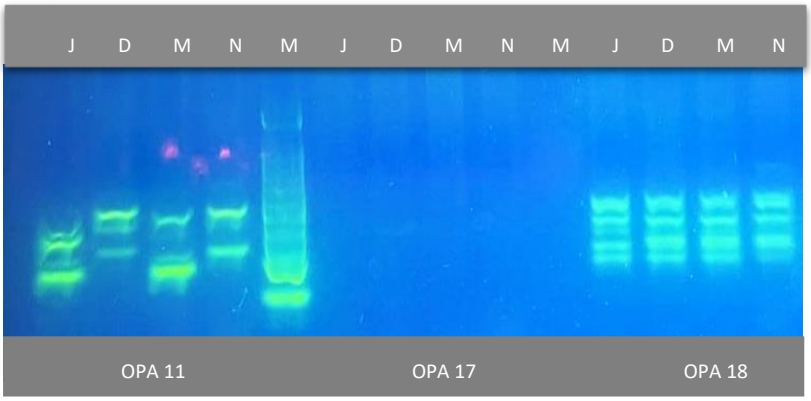

Figure 4. Visualisation of DNA bands from RAPD primers OPA 11, OPA 17 and OPA 18

Sorghum DNA bands can be seen using several primers. Of the primers used, primer OPA 18 gave the highest number of DNA bands than the other primers. While in OPA 17, the DNA bands were not clearly visible. However, all of these primers can be used in the NTSYScp programme to obtain a phylogenetic tree..

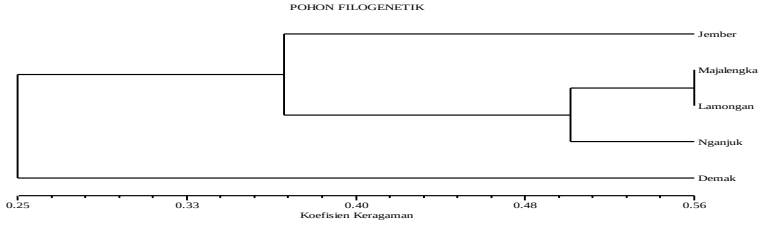

Figure 5. Phylogenetic Tree of Five Local Sorghum Genotypes with NTSYSpc programme

Based on UPGMA (Unweighted Pair-Group Method Arithmetic) using NTSYS (Numerical Taxonomy and Multivariate System) software version 2.1 can be seen in Figure 5. One factor that is very influential in developing plant breeding strategies is genetic diversity. Therefore, to breed local sorghum plants in Java, it is necessary to conduct a genetic diversity test so that it can produce superior local sorghum. Based on the results of the dendrogram, the coefficient of diversity of five local sorghum in Java. The results of the phylogenetic tree show that the local sorghum of Majalengka and Lamongan are genetically aligned, which means that the sorghum is genetically the same. Individuals in a relative group are said to be uniform if the level of genetic diversity is narrow or close [10]. While the genetic diversity of local sorghum in Demak and followed by Jember is far compared to Lamongan and Majalengka. Nganjuk local sorghum has its genetic diversity line position in the middle (Jember, Demak) and (Majalengka, Nganjuk).

CONCLUSION

From the results of the study, a phylogenetic tree of five local sorghum genotypes in Java was obtained using the NTSYSpc programme, obtained different genetic diversity. Demak local sorghum has the most distant genetic with other local sorghum. This was followed by local sorghum from Jember and then local sorghum from Nganjuk. While Majalengka local sorghum and Lamongan local sorghum have the same genetic diversity and are close to Nganjuk local.

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