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Review Article

Genetic diversity of gamma ray application result on Mandarin's SoE using ISSR markers

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Abstract

Mandarin's SoE are one of Indonesia's leading mandarin to substitute imported oranges. The quality of oranges can be improved through breeding programs, one of which is mutation breeding using gamma rays. The purpose of this study was to obtain information about the genetic variation of SoE mandarin resulting from gamma ray radiation using ISSR markers. PCR results with ISSR markers on tangerine plants produced by gamma ray radiation showed various patterns, namely the bands were the same, lost bands, and experienced the addition of new bands compared to control plants.

Introduction

Mandarin's SoE (Citrus reticulate Blanco) is one of Indonesia's superior citrus which has been designated as a national superior variety and as an imported substitute citrus, especially for the type of mandarin. Physically, oranges have fruit skins with a bright orange color, are easy to peel, have a smooth, shiny, round fruit skin texture, and are large in size (fruit diameter 7-8 cm). This orange has a distinctive taste which is a mixture of fresh sweet and sour, the color of the orange flesh, and the texture of the soft flesh. The weakness of this orange is the number of seeds that tend to be large (> 10) (Hardiyanto et al.,2007). This is certainly not liked by Indonesian consumers.

Conventional citrus breeding is mostly hampered by high juvenility in seedlings and high heterozygosity with polygenic phenotypic characters. This delay can be overcome through artificial mutation methods. The physical mutagen that is widely used for fruit plant breeding is gamma ray radiation. Gamma rays are often applied because they have the ability to penetrate deep into plant tissue. Mutation induction is directed to change one or several important characteristics that benefit plants. Artificial mutation techniques are generally aimed at changing certain characters while maintaining most of the original characters (Wardiyati et al.,2002).

To maximize mutant identification, the characterization system must be well programmed. The development of DNA markers has opened up opportunities for the rapid identification of a number of species. The use of multiple codominance markers for heterozygous species analysis is very useful because it allows individuals to be genotyped specifically (Powell et al.,1996) and is applied in various fields such as genotype selection, gene mapping, individual fingerprinting, genetic analysis of

populations, and individuals (Rafalski et al.,1996). Inter-Simple Sequence Repeat (ISSR) is a PCR-based DNA marker that uses microsatellite sequences. The advantages of ISSR markers are that their application is very simple, easy to perform, fast, involves a low quantity of DNA prints (10-30 bp), is repeatable and consistent, does not require a lot of information to design primers, and is able to distinguish individuals who are very related close (Shahsavar, 2007).

Genetic Diversity

The scope of plant breeding activities includes the formation of genetic diversity and selection. The genetic diversity that is formed can be used as a basic material or population for plant breeding processes. Increased diversity of genetic crop can be done through several ways, such as introduction, hybridization, selection, biotechnology and mutation (Pardal, 2014). The diversity of the genetic to the plant mandarin SoE can be improved by way of mutation, the mutation artificial or mutation induction.

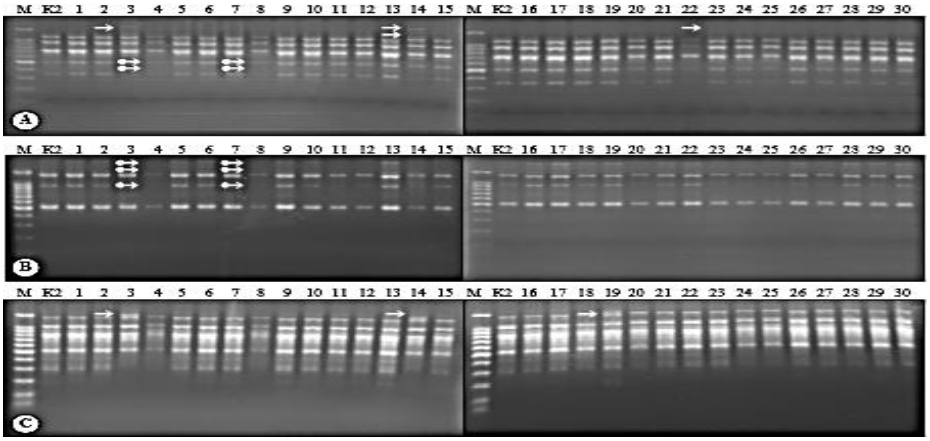
Molecular Analysis Using Gamma Ray Mutagens

The DNA material came from young leaves from a gamma ray application and a control citrus plant. Plant DNA was isolated by the CTAB method of Doyle and Doyle (1990). DNA quality testing was carried out using a spectrophotometer (Tenriulo et al., 2011). The quantity of DNA can be seen from the results of the DNA bands carried out with 1% agarose gel, then the DNA bands were documented by means of Bio Doc Analyze (Widiastuti et al., 2013).

Table 1. Primary and Total Locus Results Amplification of 3 markers ISSR

Primer	Total Locus	Polymorphic Locus	Monomorphic Locus
ISSR C (HVH(TCC)5)	8	4(50%)	4
ISSR D ((TCC)5RY)	6	4(66,67%)	2
ISSR E ((GT)8YC)	8	1(12,5%)	7
Amount	22	9(40,9%)	15

Of the five markers used, to amplify the sample DNA, three of them produced polymorphic DNA bands, and two markers produced monomorphic bands. The resulting number of polymorphic band was quite low at 40,9% (Table 1).



→ : Locus that has added a DNA band ⊷ : Locus that has lost the DNA band

Figure 1. Patterns Ribbons DNA mandarin SoE results of radiation rays gamma applied with the ISSR markers C (A), ISSR D (B) and ISSR E (C) (Farida et al., 2010).

Based on the results of DNA amplification and separation (Figure 1), the plants produced by radiation have several DNA band patterns, namely the same DNA band pattern, losing DNA bands, and experiencing the addition of new bands compared to control plants. Possible causes of band loss include deletion at the site where the primers should have attached, duplication, nitrogen base

substitution, insertion, and translocation when plant tissue is exposed to gamma rays. Ionization of bases in the DNA molecule can also cause these bases to pair incorrectly. Van Harten (1998) stated that if ionizing radiation changes DNA, gene mutations will occur. Some of the causes of the increase in bands are (1) there is deletion between the two sites where the primer attaches, (2) if there is a substitution in a certain DNA piece, it will cause a new site that is suitable for a particular primer (Harahap, 2005).

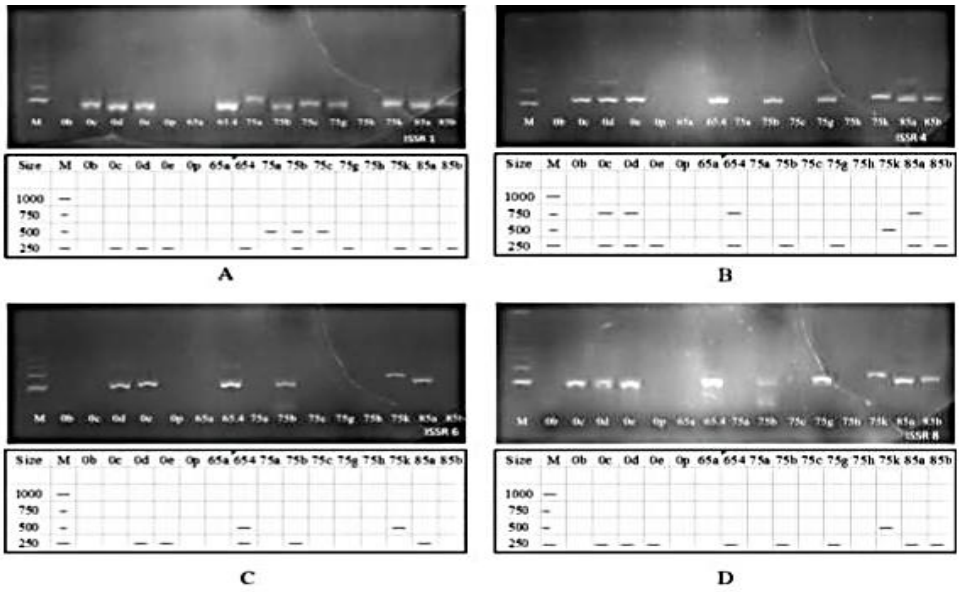


Figure 2. The pattern of the ribbon results of electrophoresis of products of PCR 15 individual samples of Test SoE mandarin using markers: A) ISSR 1, B) ISSR 4, C) ISSR 6, and D) ISSR 8. M: 1kb (Indriati et al.,2016).

The pattern of the ribbon were obtained from the results of electrophoresis of 15 samples of the products of PCR after scoring and analyzed by using Software NTSYS generate pattern ribbon diverse (polymorphic) **(Figure2**). The pattern of the ribbon are different that shows the differences make up genetic of each sample test.

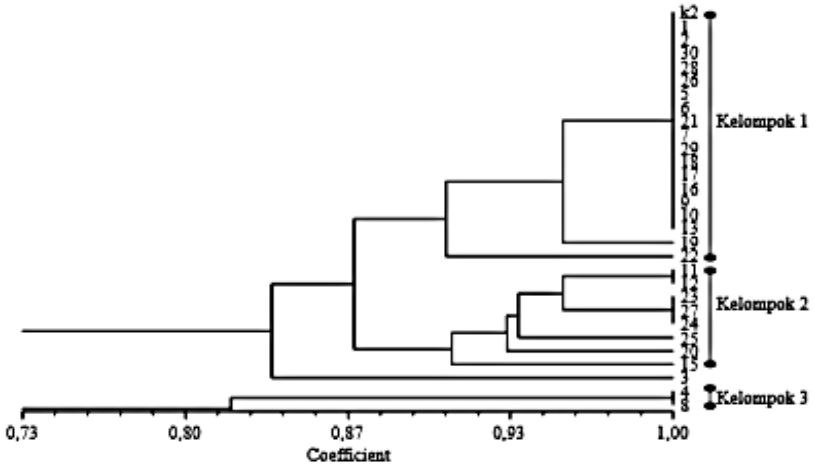


Figure 3. Dendogram orange mandarin SoE radiation result rays gamma based on the data combined markers ISSR C, ISSR D, and ISSR E (Farida et al., 2010).

Based on the resulting dendrogram, it can be seen that the Mandarin SoE resulting from gamma ray radiation have a similarity level of 73-100% or a genetic distance of 0-27% (**Figure 3**). When viewed from the genotype grouping, Mandarin SoE resulting from gamma ray radiation are not grouped based on the radiation dose, in other words, genetic grouping is not influenced by radiation dose. This is

made possible by the very individual nature of gamma ray radiation (Medina et al.,2004) and the part of cells exposed to radiation that has different mutations between plants.

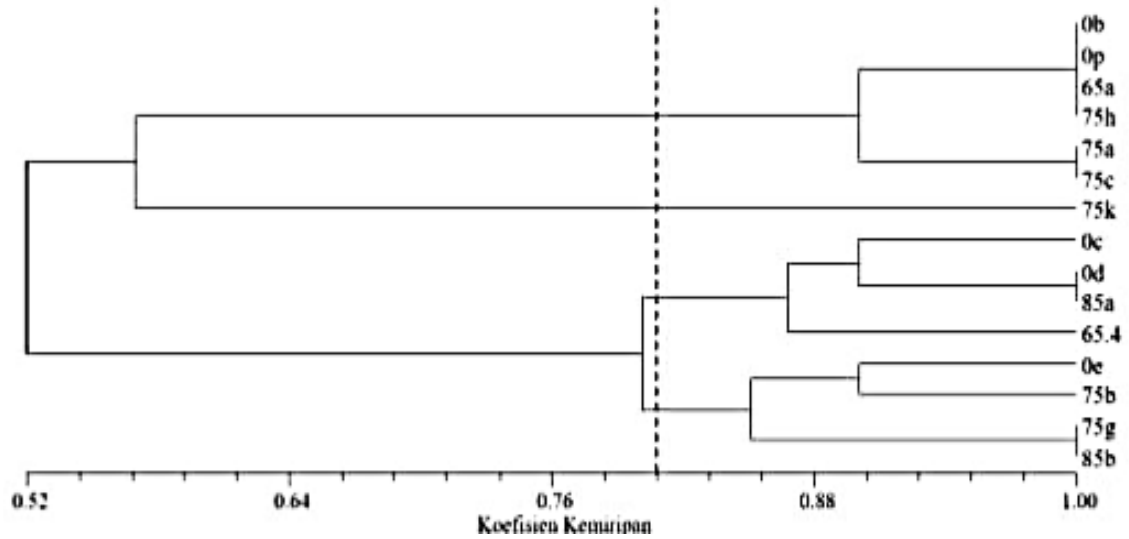


Figure 4. Dendrogram analysis of the diversity of the genetic of the sample control (0b, 0c, 0d, 0E, 0p), sample mutant hopes of generations MV1 mandarin SoE (65a, 65.4, 75A, 75B, 75C, 75g, 75h, 75k, 85a, and 85b) based on ISSR markers 1, 4, 6 and 8 (Indriati et al., 2016).

Based on the dendrogram in Figure 4 coefficient (degree of) similarity of the samples that were tested is 52-100%, with arrange of genetic 0-48%. At the level of similarity of 80% was formed four groups of individual samples of the test with the level of similarity that is the same. Individuals mutant hope 75A, 75C 90% similarity individuals 0b, 0 pand mutant hope 75h, and 50% similarity the individual 75k, so75k can be classified themselves as having a distance of genetic which is great. In group3, individual 0c was 90% similarity 0d and 85a. The two small groups were 87% similarity individuals 65.4. In Group 4, the individual 0E 90% similarity the individual mutants hope 75B, and both are similar to 85% by individuals mutant hope 75G and 85B. Individuals sampled control 0b-0p, 0c 0s, and 0E based dendrogram Figure 4 are the groups that differ indicates that individuals are already different in genetic. It that may occur due to the influence of the mutation point (point mutation) or mutation silent (silent mutation). Mutations point or mutation silent only change one partner base nucleotide without causinga change or replacement of the type of acidic amino generated or encoded from the position of three codons (Sastrosumarjo et al., 2006).

ISSR Markers

Evaluation of the diversity of genetic of mutant expectations of the results of irradiation ray gamma mandarin SoE needed to see there whether or not change the genetic that occurs in the arrangement of genes. Detection of change singene composition using PCR– based ISSR molecular markers. Marka ISSR is more appropriate to use to plant oranges, because it is not influenced by these as ons and the environment, do no need the data sequence beginning, only require 5-50mg of template DNA per reaction, spread in the whole genome, produce ribbon polymorphic up to the level of cultivars, are dominant, can be used for analysis of the diversity of genetic and analysis of kinship (Yulianti et al., 2010).

Conclusion

The conclusion of this paper is that PCR results with ISSR markers on mandarin plants resulting from gamma ray radiation show various patterns, namely the same bands, loss of bands, and experiencing the addition of new bands compared to control plants.

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