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Original Article

Molecular analysis of Karawang local soybean genotypes with national superior varieties based on SSR analysis

Análise molecular de genótipos locais de soja Karawang com variedades nacionais superiores a partir da análise SSR

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Abstract

The aim of this study was to analyze the similarities between the Karawang local superior varieties and several national superior varieties based on chromosome analysis or allele size. Analysis were carried out in the laboratory of the Biogen Research Institute in 2021. There were 9 genotypes analyzed, consisting of 8 national superior varieties, namely NS Karawang, Argomulyo, Grobogan, Anjasmoro, Devon-1, Detap-1, Dena-1 and Dena-2 and 1 Karawang local soybean genotypes. The results of the analysis show that Karawang local soybean has a difference with the national superior variety. The similarity of local soybeans from Karawang to NS Karawang with varieties Agromulyo and Grobogan is 68%, with soybean varieties Anjasmoro and Devon-1 is 71%, while with soybeans Detap-1, Dena-1 and Dena-2 is 76%.

Keywords: molecular, soybean, variety, SSR analysis.

Resumo

O objetivo deste estudo foi analisar as semelhanças entre as variedades superiores locais de Karawang e diversas variedades superiores nacionais, com base na análise cromossômica ou no tamanho do alelo. As análises foram realizadas no laboratório do Biogen Research Institute em 2021. Foram analisados 9 genótipos, constituídos por 8 variedades superiores nacionais, nomeadamente NS Karawang, Argomulyo, Grobogan, Anjasmoro, Devon-1, Detap-1, Dena-1 e Genótipos locais de soja Dena-2 e 1 Karawang. Os resultados da análise mostram que a soja local Karawang apresenta uma diferença em relação à variedade superior nacional. A similaridade da soja local de Karawang a NS Karawang com as variedades Agromulyo e Grobogan é de 68%, com as variedades de soja Anjasmoro e Devon-1 é de 71%, enquanto com a soja Detap-1, Dena-1 e Dena-2 é de 76%.

Palavras-chave: molecular, soja, variedade, análise SSR.

1. Introduction

Soybean seeds should be plant in the spring 2-3 weeks after the average last frost date, when the soil has warmed to at least 16°C. Soybean can be planted earlier in warm winter areas. Soybeans grow best where the average daily temperature is 21 ^{0}C (Amrate et al., 2023; Asadipour et al., 2005; Mojahed et al., 2022). It is better to plant soybeans in full sun. Soybean tolerates partial shade, but its yield is reduced. Soybeans grow best in loose soil with good drainage and rich in organic matter. Soybeans prefer a soil pH of 6.0 to 6.8 ( ). Soybean is resistant to poor soil. Varieties play an important role in the development of planting, because achieving high productivity is largely determined by the yield potential of the superior varieties planted. Yield potential in the field is also influenced by the interaction between genetic factors of varieties and growing environmental conditions (Kumar et al., 2022). If the management of the growing environment is not carried out properly, the high yield potential of these superior varieties cannot be achieved (Abdel-Baky et al., 2023; Nodar et al., 2022; Jamalpour and Derabi, 2023). According to Rey et al., (2022) in Shariati et al., (2013), the ideal superior variety is high yielding, resistant to major pests and diseases and stable in various environments.

Superior varieties have an important position as an increase in the productivity of soybean yields. With the availability of various superior varieties of soybeans, it is hoped that farmers will re-plant soybeans to meet the needs of West Java and/or nationally, which are currently still far greater than their production capacity. Superior soybean varieties for one area may not necessarily show

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the same superiority in other areas, due to differences in climate, topography and planting methods. The use of location-specific superior variety seeds is an alternative in efforts to increase soybean production. In order to increase the availability of superior seeds, various efforts have been made in the seed sector, including by releasing new superior varieties, both locally and nationally, as a result of plant breeding (Habtegebriel and Abebe, 2023; Sherafatizangeneh et al., 2022; Ali et al., 2022). There are more than 70 soybean varieties that have been released and disseminated to farmers, but it is necessary to study the level of adaptation and stability in various agro-ecosystems such as irrigated paddy fields, rain-fed paddy fields and land so that varieties with high yields are known in these agro-systems (Amrate et al., 2023; Farhud and Mojahed, 2022). New superior varieties can be produced, among others, through purification/bleaching from local varieties that have long been known and favored by farmers.

In Karawang there is potential for paddy fields which are quite large and can be utilized for soybean area expansion programs in an effort to increase production. The white flower local variety is a type of local soybean that has long been circulating in the community, especially in Karawang regency. Karawang Putih Bunga Putih Local Soybean is a local variant that is favored by farmers because of its large seeds and high production. Karawang farmers usually harvest the young to make boiled soybeans. Karawang Local Soybeans circulating in the community are still not uniform because the seeds planted are mixed and obtained in the traditional way which does not use seed propagation methods. From the results of purification carried out by the Karawang Regency Agriculture Office and the Center for Supervision and Certification of Food Crops and Horticulture Seeds of West Java Province for local Karawang local soybeans, it turns out that there are 2 types of variants. The Likal Karawang soybean variety, the purple flower variant, has received a Release Decree from the Ministry of Agriculture in 2017 under the name NS Karawang Variety, while the white flower variant has not been identified as to whether it is a different type from the existing variety and was released by the Ministry of Agriculture.

To obtain superior Karawang local varieties with white flower variants, observations are still needed which will be carried out in production centers in Karawang Regency, with appropriate or adaptive agro-ecosystem conditions to be compared with superior varieties that are widely planted by the community to determine their superior characteristics. The national superior soybean varieties that people are interested in today are the Anjasmoro variety, the Argo Mulyo variety, the Orba variety, and the Rajabasa variety (Maranna et al., 2023; Gholivand et al., 2021b). Soybean varieties with different genetic backgrounds will look different when grown in the same environment, because appearance is the result of interaction between genetics and the environment (Bhuiyan et al., 2022; Tightiz and Yoo, 2022). Based on this, the characteristics of the local white flower Karawang variety will be different.

The development of biotechnology allows breeders to utilize a tool in the form of molecular markers to assist identification. Molecular markers refer to differences in sequences or sequences of Deoxyribo Nucleic Acid (DNA), which are the main constituent elements of genes. This difference is able to distinguish one individual from another specifically. The basic concept of molecular markers is that one individual has a DNA sequence that is unique to other individuals so that these differences can be used as markers. The advantage of DNA markers in conducting selection is that they are stable, not affected by the growth phase or environmental conditions (Fernandes et al., 2022; Ghaderloo et al., 2023; Grosu et al., 2021).

The evaluated morphological characteristics include the bark and leaf structures of endemic plants. Molecular characterization was carried out by sequencing of matK, ITS, and trnL-trnF genes (Lukman et al., 2022). According to Sumarno and Zuraidah (2004) and Najafi and Nasiri, (2019) the more germplasm collections, the greater the chance to get superior varieties. Collection of potential local rice germplasm needs to be continuously carried out because the availability of germplasm which has a large variety is a source of genes that support efforts to form new varieties that have high yields, resistance to pests or diseases, early maturity and other characteristics (Sitaresmi et al, 2013). The aim of this study was to identify the differences between local Karawang white flower soybeans and several national superior varieties by using molecular analysis Simple Sequence Repeat (SSR).

2. Research Methods

The white flower Karawang local variety is planted in paddy fields in Tamanmekar Village, Pangkalan District, Karawang Regency, West Java Province According which is located at an altitude of 85 meters above sea level and the soil type is Grumosol (BP3K Pangkalan District, 2010). The climate type for Tamanmekar Village, Pangkalan District, Karawang Regency is type C (Slightly Wet) (Yao et al., 2023; Gholivand et al., 2021a). The plant materials of this research were 144 soybean genotypes from different ripening groups (early, medium and late) which were obtained from the Biogen Research Institute gene bank. Favorable climatic for soybeans seed are the ambient temperature at 25°C with a humidity of 13% to 13.3% at a relative humidity of 65%.The studied genotypes were cultivated in the Pangkalan Seed and Breeding Research Institute in 2021 in the form of a simple lattice design with two replications. It should be noted that the seeds of 14 genotypes did not germinate due to various reasons, so this project was implemented with 130 genotypes. The control varieties were the NS Karawang variety (purple flower), Anjasmoro variety, Argomulyo variety, Orba variety, Grobogan variety, and Rajabasa variety. Molecular analysis using the Simple Sequence Repeat (SSR) method was carried out at the Balitbiogen Laboratory in Bogor to find out the differences between the local Karawang white flower variety and the control variety.

3. Research Result

The gene arrangement of individuals will remain the same at various growth phases and in different environmental conditions. This makes DNA markers a good tool to assist the process of carrying out the identification and analysis of differences. Various DNA markers have been used in the selection process, including Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Inter-SSR (ISSR), Amplified Fragment Length Polymorphism (AFLP), transposon-based markers, to Single Nucleotide Polymorphisms (SNPs). These markers have been widely used and have yielded significant results for improving the properties of various crop commodities such as yield, quality, and resistance to drought stress, salinity, and pest attack. Based on its advantages, DNA markers are a tool that has the potential to be utilized in increasing the efficiency and effectiveness of the selection process to improve plant properties through plant breeding or identifying new varieties and observing different characters (Agus, 2019). DNA barcoding has been used as a powerful tool and a practical method for species characterization and delimitation.

The present work aims to evaluate molecular markers for barcoding three Piper species native to Brazil: P. gaudichaudianum ("jaborandi" or "pariparoba"), P. malacophyllum ("pariparoba-murta") and P. regnellii ("caapeba" or " pariparoba).

The Malva genus contains species that reveal therapeutic properties and are mostly important in medicine and the functional food industry. Its breeding, cultivation, and utilization are based on proper germplasm/plant identification, which is difficult using morphological features. For this reason, we applied flow cytometry and inter simple sequence repeat polymerase chain reaction (ISSR-PCR) for fast and accurate species identification (see Figure 1). (Jedrzejczyk and Rewers, 2020). The study aimed to get the molecular and morphological identity of patchouli (Pogostemon sp.) from BPP Batang Regency. Amplification of ITS fragment patchouli produced a 670 bp-sized single band. Phylogenetic analysis showed patchouli BPP related to Pogostemon cablin (KR608752.1) with 98% coverage identity (Yudifian et al., 2022). Identify the agarwood-producing plant species in North (Jedrzejczyk and Rewers, 2020). The study aimed to get the molecular and morphological identity of patchouli (Pogostemon sp.) from BPP Batang Regency. Amplification of ITS fragment patchouli produced a 670 bp-sized single band. Phylogenetic analysis showed patchouli BPP related to Pogostemon cablin (KR608752.1) with 98% coverage identity (Yudifian et al., 2022). Identify the agarwood-producing plant species in North (Jedrzejczyk and Rewers, 2020).

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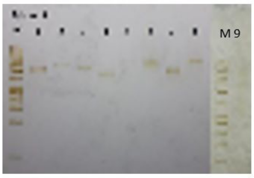
A number of SSR markers (Table 1) had a significant relationship with several traits, which can be caused by pleiotropy effects or the continuity of genomic regions in the control of these traits. The association of several markers with one trait or indicating the quantitative and polygenic inheritance of these traits, each of which controls a specific share of the diversity of the trait. By examining genetic diversity in different soybean genotypes through SSR markers, the average allelic frequency was 0.31, the average number of ALD was 53.5, the average number of effective ALD was 62.4, and the average PIC was 0.73. Nei was estimated as 0.77 and Shannon's average was 1.57. The ideal superior variety is high yielding, resistant to major pests and diseases and stable in various environments. Based on these factors, white-flowered Karawang local variety is superior.

As shown in Figure 2, the the local Karawang white flower soybean has a genetic similarity with purple flower soybean by 68%. The similarity of white flower soybean with Agromulyo and Grobogan varieties is 68%, with Anjasmoro and Devon-1 varieties is 71%, while with Detap-1, Dena-1, and Dena-2 soybeans is 76%. Also it can be said that purple flower soybeans have genetic similarity with Anjasmoro soybeans by 66%, with Devon-1 by 67%, with Detap-1 and Dena-1 by 68%, with Dena-2 by 9%, with Agromulyo by 72%, with Grobogan 65%.

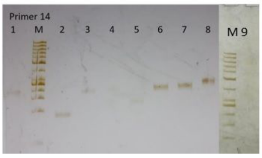
Table 1. SSR analysis results of genetics.

Variety	Anjasmoro	Devon- 1	Detap-1	Dena-1	Dena-2	Purple	Agromulyo	Grobogan	White
Anjasmoro	1.00								
Devon-1	0.77	1.00							
Detap-1	0.78	0.73	1.00						
Dena-1	0.79	0.73	0.79	1.00					
Dena-2	0.74	0.74	0.75	0.75	1.00				
Purple	0.66	0.67	0.68	0.68	0.69	1.00			
Agromulyo	0.69	0.69	0.68	0.69	0.70	0.72	1.00		
Grobogan	0.70	0.68	0.72	0.72	0.73	0.65	0.66	1.00	
White	0.71	0.71	0.76	0.76	0.76	**0.68**	0.68	0.68	1.00



No	variety	Allele Size
1	Anjasmoro	617 pp
2	Devon-1	595 pp
3	Detap-1	456 pp
4	Dena-1	687 pp
5	Dena-2	660 bp
6	Purple	524 pp
7	Agromulyo	695 pp
8	Grobogan	526 pp
9	White	-

Primer Satt009

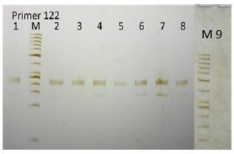


No	variety	Allele Size
1	Anjasmoro	205 pp
2	Devon-1	96 bp
3	Detap-1	227 pp
4	Dena-1	229 pp
5	Dena-2	153 pp
6	Purple	268 pp
7	Agromulyo	277 pp
8	Grobogan	315 bp
9	White	-

Primer Satt014

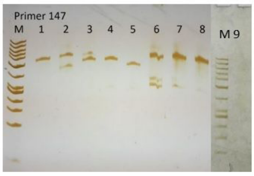
No	variety	Allele Size
1	Anjasmoro	95 bp, 63 bp
2	Devon-1	96 bp, 62 bp
3	Detap-1	96 bp, 68 bp
4	Dena-1	100 bp, 66 bp
5	Dena-2	104 bp, 68 bp
6	Purple	527 bp, 65 bp
7	Agromulyo	66 bp
8	Grobogan	67 bp
9	White	-

Primer Satt038



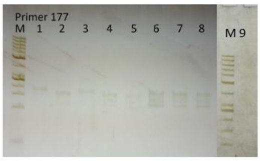
No	variety	Allele Size (bp)
1	Anjasmoro	224, 152
2	Devon-1	208, 143
3	Detap-1	227, 153
4	Dena-1	226, 154
5	Dena-2	233, 148
6	Purple	242, 167
7	Agromulyo	246, 166
8	Grobogan	241, 162
9	White	-

Primary Satt122



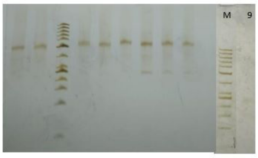
No	variety	Allele Size (bp)
1	Anjasmoro	605
2	Devon-1	659, 486
3	Detap-1	745, 635
4	Dena-1	630
5	Dena-2	529
6	Purple	710, 362, 299
7	Agromulyo	728, 293
8	Grobogan	684, 264
9	White	-

Primary Satt147



No	variety	Allele Size (bp)
1	Anjasmoro	175
2	Devon-1	157, 113
3	Detap-1	172, 139
4	Dena-1	152, 128, 93
5	Dena-2	148, 107
6	Purple	166, 135, 115
7	Agromulyo	161, 133, 112
8	Grobogan	152, 124, 107
9	White	-

Primary 177



No	variety	Allele Size (bp)
1	Anjasmoro	478, 334, 225
2	Devon-1	499, 337, 221
3	Detap-1	573
4	Dena-1	552
5	Dena-2	601
6	Purple	585, 402, 249
7	Agromulyo	532, 376, 230
8	Grobogan	537, 411, 230
9	White	215

Primary Satt242

No	variety	Allele Size (bp)
1	Anjasmoro	470
2	Devon-1	484
3	Detap-1	474
4	Dena-1	486
5	Dena-2	462
6	Purple	453, 253, 201
7	Agromulyo	464, 256, 201
8	Grobogan	472, 262, 211
9	White	221, 276

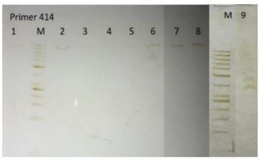
Primary satt243

No	variety	Allele Size (bp)
1	Anjasmoro	707
2	Devon-1	773
3	Detap-1	726, 298
4	Dena-1	873
5	Dena-2	850, 745
6	Purple	850, 330
7	Agromulyo	930, 422, 358
8	Grobogan	859
9	White	-

Primary satt294

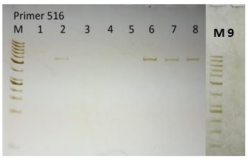
No	variety	Allele Size (bp)
1	Anjasmoro	247
2	Devon-1	264
3	Detap-1	273
4	Dena-1	-
5	Dena-2	293
6	Purple	318, 230, 190
7	Agromulyo	307, 185
8	Grobogan	387
9	White	-

Primary Satt308



No	variety	Allele Size (bp)
1	Anjasmoro	971
2	Devon-1	940
3	Detap-1	956
4	Dena-1	921
5	Dena-2	999
6	Purple	956
7	Agromulyo	987
8	Grobogan	1066
9	White	436, 346

Primary Satt414



No	variety	Allele Size (bp)
1	Anjasmoro	747
2	Devon-1	583
3	Detap-1	722
4	Dena-1	747
5	Dena-2	737
6	Purple	583
7	Agromulyo	586
8	Grobogan	578
9	White	-

Primary Satt516

Figure 1. Variants of genetics.

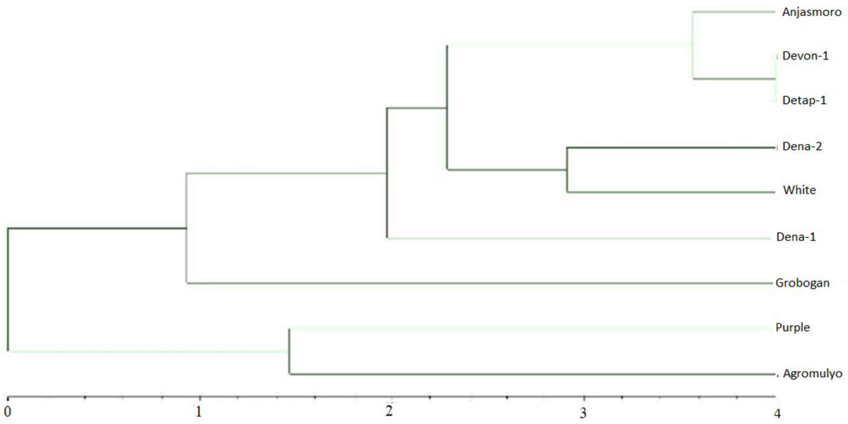


Figure 2. Dendrogram derived from clustering analysis.

4. Conclusions

In general, the results showed that in addition to using markers as useful markers in the evaluation of genetic diversity in soybean, markers that had more connections with the genomic regions controlling these traits are introduced to improve the population through selection with the help of markers. Based on the results, it was suggested that the white-flowered Karawang local variety was submitted for release, starting with testing for yield and resistance to the main pests with a comparison of the high-yielding varieties used as a comparison in this study. Considering that the studied SSR sites were effective on the studied traits, it is possible that these gene sites can be used in breeding programs to identify parents to prepare mapping populations and also to produce hybrids.

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