Phylogenetic Analysis Of Salt Resistant F4 Progeny Of Soybean (Glycine Max (L.) Merril) With Using SSR (Simple Sequence Repeats) Marker

Evi Julianita Harahap, Rosmayati, Diana Sofia Hanafiah

Abstract: Phylogenetic is one of the most commonly used methods in systematics to understand the diversity of living things through the reconstruction of kinship relationships (phylogenetic relationship). SSR marker has some merits such as quickness, simplicity, rich polymorphism and stability, thus being widely applied in genetic diversity analysis, molecular map construction and gene mapping. UPGMA method of phylogenetic analysis is normally the sum of the identical sequences. The objective of this research to find out the genetically diversity and identification of F4 progeny soybean genotype of carrier character salt resistant with used SSR (Simple Sequence Repeats) marker. This research was conducted in Bio molecular Laboratory, Socfindo Seed Production Laboratory (SSPL), Kebun Bangun Bandar Village Martebing District Dolok Masihul Regency Serdang Bedagai on December-May 2017. The number of samples were used 44. Analysis of the data were used software Microsoft Excel 2007, GenAlex ver.6.501, and Multivariate Statistical Package (MVSP) ver.3.2. The UPGMA phylogenetic analysis identified to bring female of 11 plants, male of 3 plants, and both were male and female of 7 plants with coefficient of genetically suitable 0,52.

Index Terms: Progeny F4, soybeans, SSR, saline resistant, phylogenetic

1. INTRODUCTION

Soybean is a very important crop. Soybean has a high oil content, and the seeds are rich in protein. Its uses include for the manufacture of tempeh, tofu, milk, flour, oil, cosmetics, soap, food products, pharmaceuticals, fertilizers, paint industry, varnish, plastics, and others [1]. Efforts to increase soybean production currently face constraints in the form of decreasing planting area and depletion of fertile land due to land conversion to non-agricultural sector. Optimization of domestic soybean supply is likely to be directed to suboptimal land, including saline area. One strategy to overcome and eliminate the decrease of soybean production is to assemble salinity tolerant varieties [2]. The field selection and field tests for morphological characters have been generally used by plant breeders to describe varieties, but require considerable time and most of the visible characteristics of genetic interactions and environmental conditions [3]. Through the application of breeding strategies that incorporate molecular biology approaches can be identified important properties that will be glorified at the DNA level. Molecular analysis is needed to help the process of plant selection more quickly and accurately. Use of molecular markers is expected to improve the accuracy of the selection process and improve the efficiency of selection time [4]. The SSR marker is a molecular marker that will detect a series of nucleotide patterns (DNA), usually between two to six pairs of N bases, which are repeated sequentially. Some of the advantages of SSR techniques are high polymorphism, codominant, large number of loci, and does not take long [5].

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According to [6] conducted a validation study of some salinity-resistant soy cultivars with SSR markers. His research used 5 SSR markers: QS08064, QS080465, QS1101, QS1112, and QS100011. The results show that the QS08064 marker is the best SSR marker among others used in 35 genotypes of salinity tolerance and 23 sensitive soybean accessions. This marker shows the selection efficiency of 76.2% to 94.2%. The objective of this research to find out the genetically diversity and identification of F4 progeny soybean genotype of carrier character salt resistant with used SSR (Simple Sequence Repeats) marker.

2 MATERIAL AND METHODS

The research was conducted in December 2016-May 2017 at the Biomolecular Laboratory, Socfindo Seed Production Laboratory (SSPL), Bangun Bandar Village Martebing Village Dolok Masihul Serdang Bedagai District. This study used the root of F4 progeny from female elder crosses (Grobogan Varieties (G)) with salt-resistant male genotypes. The number of samples used are 44 samples consisting of progeny population 40 individuals, female elder population 2 individuals, and male elder population 2 individuals. Each individual is numbered for ease of tagging, individuals 1-40 are progeny, 41-42 are female elders, and individuals 43-44 are male elders.

2.1 DNA Extraction

The DNA isolation was taken from the root of 40 F4 soybean progeny, 2 male elders and 2 female elders with a total of 44 samples. The procedure of DNA isolation from CTAB method by [7] modifications. DNA quantity testing was performed using nanophotometers at wavelength (λ) 260 nm and 280 nm using 2 µl of total DNA isolated and purified.

2.2 School Environment

School is a formal educational environment, because there are curriculum in schools as education and teaching plan, more professional teachers, facilities and special educational facilities to support educational process and special education.

School environment also plays an important role for the development of student learning. The school environment includes: the physical environment of the school such as, learning facilities and infrastructure, learning resources, and learning media, social environment, concerning the relationship of students with their friends, teachers and other school staff, the academic environment such as the school building, the implementation of teaching and learning activities, etc (6).

2.3 Teachers' Attitude

A teacher should have a good attitude in teaching activities. Attitudes are either pleasant or unpleasant evaluative statements about objects, people, or events (2). Attitudes are a determinant of behavior, because they are related to perception, personality, and motivation (7). There are three components of attitude stated as follows:

2.4 PCR and Electrophoresis

PCR amplification using 5 SSR markers was run on the basis of research by [6] that has been modified. The amplification program consists of a 4 minutes predenaturation cycle at 950C for 10 minutes, 35 denominations of 940C for 30 seconds, 35 annealing cycles 550C for 1 minute 15 seconds, 35 elongation cycles 720C for 1 minute 30 seconds, and final cycle extension 720C for 30 minute, and incubation of PCR 40C. Electrophoresis of PCR amplification results was done on 2% agarose gel. The gel was prepared by dissolving 1.6 g of agarose and 80 ml of TAE 1x added gelred ethidum bromide 0,5 µl dye. Electrophoresis is run on 50 watts which is powered by 110 volt and 25 mA for 30 minutes. The electrophoresis results were observed and documented with UV-transilluminator (UV Doc-its) and Gel-Doc (U Doc-its).

2.5 Data Analysis

Data analysis based on the scores of DNA band patterns appearing on agarose gel. The disked tape in the form of binary data with no band (1) or absent (0) ribbons is assisted by using GenAlex's excel software add-ins ver.6.501 [8]. To determine the value of Polymorphic Information Content (PIC) based on the formula PIC = 1 - ΣPij2. where Pij is the frequency of the allele j-th for the i-th locus that satisfies all alleles for the locus. Phylogenetic analysis of UPGMA and calculating genetic distance between individuals used Multivariate Statistical Package (MVSP) software ver.3.2

3. Result and Discussion

3.1 Teachers' Attitude

The results of analysis with 5 primers on saline resistant character obtained the number of alleles per primer with a diverse range. The number of alleles per primer ranged from 8 to 13 alleles (Table 1).

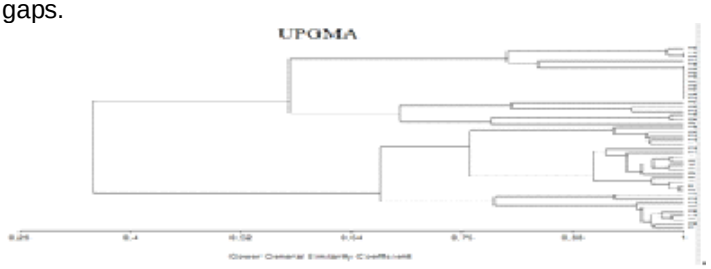
Table 1. *Profile of 5 SSR Markers on 44 individuals against salinity resistant characters*

No	Primary Name	Number of Detected Alleles	Percentage of Polymorphism (%)	PIC
1.	QS080465	12	100	0,87
2.	QS1101	9	100	0,83
3.	QS1112	8	100	0,86
4.	QS100011	10	100	0,83
5.	Sat_091	13	100	0,89
	Means	10,4	100	0,86

The highest number of alleles is found on Primer Sat_091 as many as 13, while the lowest number of alleles is found in primary QS1112 only 8. The percentage of polymorphisms of the five primers is 100%. This suggests that the primer is a polymorphic specific primer for saline resistant characters. The high percentage of primary polymorphisms suggests that the primary ability to amplify the target sequence. [9] states that polymorphism is a picture of amplification obtained from differences in observed and discordable DNA fragments as the presence or absence of sequence differences to indicate the presence or absence of variation. Polymorphic Informative Content (PIC) of 5 primers ranged from 0.83-0.89 with a mean of PIC of 0.83. The highest PIC value is found on Primary Sat_091 at 0.89 and lowest on primary QS1101 and QS100011 (Table 1). Based on these results it can be seen that the five primers are very informative. [10] suggest that the amount of genetic diversity in the population is determined by the number of genes that have more than one allele (polymorphic genes), and the number of alleles in each of these genes. [11] classifies the PIC values into 3 classes: PIC> 0.5 (very informative), 0.25> PIC <0.5 (medium), and PIC <0.25 (low).

3.2 Phylogenetic Analysis of Soybean F4 Against Resistant Salt Resistant

Phylogenetic results with UPGMA (Figure 1) showed that the genetic coefficient of coefficient for all individuals tested ranged from 0.36 to 1.00. In the genetic equivalence coefficient 0.52 all the individuals analyzed were divided into 2 clusters. Cluster I consists of 19 individuals consisting of 9, 12, 14, 17, 21, 22, 23, 24, 25, 26, 27, 30, 31, 32, 33, 34, 35, 36, and 40; and Cluster II numbered 21 individuals consisting of numbers 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 13, 15, 16, 18, 19, 20, 28, 29, 37, 38, and 39. The progeny that was in the same group with the female elders of 11 plants are numbered 1, 2, 3, 6, 13, 16, 18, 28, 29, 37, and 38. This indicates that the progeny has a closer character to the elders high yielding female. Progeny closer to the male elders of 3 plants were 5,7, and 8. This indicates that the progeny carries a saline-resistant character derived from the male elder's character and this can be used for subsequent cultivation of F5. Progeny closer to the two elders of 7 crops were progeny number 4, 10, 11, 15, 19, 20, and 39. [12] states that genotypes originating from the same region are not always in the same cluster. That is, the diversity of geography does not always have anything to do with genetic diversity. [13] states that this method normally calculates a similarity scores defined as the total number of identical sequences and the number of conservative substitutions in the sequence of two sequences with negligible gaps.



4 Conclusion

The results of this study indicate that the genetic diversity of soybean F4 progeny based on polymorphism information content (PIC) was > 0.5 (very informative) on all markers

(QS080465, QS1101, QS1112, QS100011, and Sat_091) used with a mean of 0.83. Based on the phylogenetic UPGMA shows the identified progeny carrying the female elder of 11 plants, carrying the male parent characters as much as 3 plants, and the progeny that brought the second character of the parents as many as 7 plants. The soybean F4 progeny number that carries the male character of the copy-proof male parent was numbered 5, 7, and 8.

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