RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
53 (1) 126-138, 2021

GENETIC ANALYSIS OF SOYBEAN MUTANT LINES RESISTANCE TO STEM ROT DISEASE (*Athelia rolfsii* Curzi)

D.S. HANAFIAH[1*], I. SAFNI[1], L.A.M. SIREGAR[1], R.I.M. DAMANIK[1] and A. LESTAMI[2]

[1]Department of Agrotechnology, Universitas Sumatera Utara, Medan, Indonesia
[2]Postgraduate in Agrotechnology Magister, Universitas Sumatera Utara, Medan, Indonesia
*Corresponding author email: diana.hanafiah@usu.ac.id
Email addresses of coauthors: irda@usu.ac.id, luthfi1@usu.ac.id, revandy.iskandar@usu.ac.id, lestami.anggria@gmail.com

SUMMARY

Soybean is an important food and trade commodity that is extensively cultivated all over Indonesia. The stem rot disease caused by soil fungus (*Athelia rolfsii* Curzi) is responsible for significant yield losses. Enhanced soybean production can be achieved and maintained by using superior genotypes with resistance to stem rot disease. In soybean, resistance to stem rot can be incorporated in newly developed genotypes through mutation breeding. The aim of the present research was to determine the genotypic differences regarding soybean mutant lines having resistance to *A. rolfsii* in comparison to parental genotypes and control. Five spesific simple-sequence repeats (SSRs) primers (Satt009, Satt114, Satt147, Satt191, Satt197) for resistance to soybean stem rot were used to analyze eight M_6 mutant lines in comparison to parental genotype 'Anjasmoro' (parental) and resistant cultivar 'KipasPutih'. All the recorded data were analyzed through molecular analysis using Power Marker 3.25, Gen Alex 6.501 and DARwin software. Results revealed that mutant line M200-A17 (13/6) was identified as resistant line to *A. rolfsii*. Thismutant line had the same DNA marker profile as the soybean resistant genotype KipasPutih. Therefore, it was concluded that M_6 line M200-A17 (13/6) has the potential of resistance to stem rot disease and can be used as source material to develop resistant genotypes in soybean.

Keywords: Soybean, stem rot disease (*A. rolfsii*), mutant lines, mutation breeding, molecular analysis

Key findings: Genetic and molecular analyses revealed a soybean mutant line that was resistant to fungus *A. rolfsii* , which has the potential to be used in breeding for developing the superior soybean genotypes.

Manuscript received: July 27, 2020; Decision on manuscript: February 2, 2021; Accepted: February 10, 2021.

Communicating Editor: Dr. Aris Hairmansis

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is one of the most important food commodities after rice and maize (Rahmat *et al*., 2018). Based on past findings, it is estimated that the soybean production and consumption will experience an increase in the deficit during 2016 to 2020 with an average of 36.95% per year in Indonesia. During 2016 to 2020, the shortage in soybean supply was 1.60, 1.78, 1.84, 1.92 and 1.91 million tons, respectively (Nuryati *et al*., 2015).

Soybean yield losses mostly occur due to stem rot disease (*Athelia rolfsii* Curzi). Stem rot disease (*A. rolfsii*) is a teleomorphic form of *Sclerotium rolfsii* Sacc which has an inverted basidiocarp shape along with hypha arising from the sclerotia body which made a taxonomic revision of *S. rolfsii* to *A. rolfsii* (Tu and Kimbrough, 1978). Field studies exhibited that *A. rolfsii* infection in susceptible legumes reduced the pod yield by 74% in peanut (Rani, 2001). The *A. rolfsii* resistance character identified in soybean mutant lines is suspected because of a plant defense system in form of secondary metabolite content. In gamma-rays irradiation, the free radicals are generated which act as stress signals and stimulate the stress response by increasing the synthesis of polyphenol acids which acts as antioxidative defense in lettuce and other crop plants (Fan *et al*., 2003).

In soybean mutant lines, the resistance to *A. rolfsii* is suspected to have higher content of secondary metabolites compared to susceptible genotypes, and secondary metabolites are toxic to pathogens. Secondary metabolites pay role in resistance to predators and pathogens (Croteau *et al*., 2000). Plants that are capable of producing secondary metabolites have the potential to be used as a source of resistant genes to certain pests and diseases (Leiss *et al*., 2011). Prats *et al*. (2013) reported that in resistant genotypes the phenolic extracts showed growth inhibitory activity to the fungus *Sclerotinia ascospores* and were found stronger than susceptible genotypes in sunflower.

Breeding efforts have primarily focused on increasing yield first, before attempting to incorporate disease resistance traits. Furthermore, trade-off can be expected when breeding exclusively made for disease resistance due to associated energy requirements that may limit yield and metabolic activities in rice (Wang *et al*., 2015). The trade-off has also been observed historically when attempting to improve multiple traits simultaneously in soybean, which further complicates breeding efforts (Recker *et al*., 2014). Therefore, continuous evaluation of desirable soybean genotypes for various traits is necessary for identification and development of elite soybean breeding lines.

Use of resistant genotypes is the most effective way to reduce soybean yield losses, however, resistance is rare. Various type of efforts are required to create superior soybean cultivars with disease resistance and high productivity. According to Acquaah (2007), utilization of genetic resources through breeding programs to obtain superior lines of early-middle age with disease resistance and high yield through irradiation tools is one of the promising breeding approaches. This statement was evident from the findings of Hanafiah *et al*. (2010) which enunciated that variation obtained among the M1 and M2 lines for various traits influence plant growth and development either through qualitative and quantitative nature which finally improve the plant's production.

Genetic variability among the populations caused by mutation and hybridization breeding is the basic criteria needed for the selection process. However, in the populations the level of diversity will decreases along with the selection from generation to generation. Genetic diversity within the population will decrease because of high homozygosity, however, the diversity among lines is expected to be high to have the

opportunity to choose the desired soybean lines (Asadi and Dewi, 2016).

In soybean, the likelihood of success in breeding is determined by the existence of wide genetic diversity in germplasm. In particular, cultivars with greater genetic potential could play an important role in soybean production (Marliah *et al*., 2012). Morphological evaluation and molecular characterization have been carried out by the breeders time to time. However, morphological characterization is strongly influenced by the environment and requires a long time evaluation and confirmation in the field, therefore, molecular study is recommended in watermelon (Solmaz *et al*., 2010).

Presently, the integration of genomics and molecular-based breeding strategies for developing disease resistance, with gene-based marker assisted selection (MAS) being particularly effective, and a powerful method for efficient selection. In this context, pyramiding several major resistant genes into a valuable genetic background is simplified by the use of marker-based selection (Song *et al*., 1995). Molecular markers are more effective for breeding and intensive selection because the conventional breeding might take several years (Cardona *et al*., 2018). Therefore, the present study aimed for screening of several soybean M6 mutant lines and confirmation of resistant genes to stem rot disease (*A. rolfsii*) via SSR markers.

MATERIALS AND METHODS

Plant material

Soybean cultivar Anjasmoro was used as parental genotype for irradiation in this study. Anjasmoro is a superior soybean cultivar with larger seeds and resistant to lodging released during 2001 in Indonesia (Suhartina, 2005). Anjasmoro soybean seeds were irradiated with gamma rays with three different doses of 100, 200, and 300 Gy to obtain soybean populations with some desired characteristics, including resistance to stem rot (*A. rolfsii*).

A total of eight individual M_6 soybean mutant lines were obtained from the M_5 generation, namely M100-A25(5/3), M100-A6(31/1), M200-A11(32/3), M200-A12(6/5), M200-A17(13/6), M200-A17(18/5), M300-A6(33/8), and M300-A8(35/7). The cultivar Anjasmoro was used as as parent and cultivar KipasPutih was used as check (as comparative resistant to stem rot disease (*A. rolfsii*). The said research was conducted during February to April 2019 at the Field and Laboratory of Plant Biotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.

Phenotypic observations and data analysis

The seeds of all the soybean mutant lines along with parental and check genotypes were grown in two different environments i.e., a) soil treated with inoculation *of A. rolfsii*, and b) soil without inoculation of *A. rolfsii* having plot size of 2.5 x 13 m. The planting-hole was made using the tip of a finger, with a plant spacing of 30 x 15 cm. Soybean seeds were planted in holes with one seed per hole and subsequently covered with top soil. Fertilizer was applied at seven days after planting according to recommended dose for soybean; Urea 2.3 g/planting hole, SP-36 3.4 g/planting hole, and KCl 4.5 g/planting and applied 7-10 cm around the hole of the plant.

The experiment was arranged in an augmented design where the M_6 genotypes were planted in rows without replication, and the parental and check genotypes were planted with replications. The parental cultivar Anjasmoro and check genotype KipasPutih were grown with a total of 42 plants per replication. Meanwhile, the eight groups of genotypes comprised of 56 plants each. Data on single plant basis were collected and then averaged for each character, and then the mean values for each population were tested by using t-test (Sharma, 2006).

DNA Extraction

DNA extraction was made from the leaves of soybean mutant lines aged of two weeks after planting (WAP), individually. For DNA extraction, the cetyltrimethyl ammonium bromide (CTAB) method by Orozco-Castilo (1994) with addition of polynillpolypirrolidone (PVPP) and β-mercaptoethanol was followed (Toruan-Mathius and Hutabarat, 1997; Asmono *et al*., 2000). DNA quantity testing was carried out by the spectrophotometer method (Gallagher and Desjardins, 2006), while DNA quality test was made through 2% agarose gel using electrophoresis and visualization methods with UV-Tex (Lee *et al*., 2012).

Polymerase chain reaction (PCR)

Amplification was carried out using five SSR primers namely Satt009, Satt114, Satt147, Satt191, and Satt194 (Arahana *et al*., 2001). The amplification program consisted of four minutes pre-denaturation cycle at 94ºC, followed by 35 denaturation cycles of 94ºC, for 30 seconds, annealing 52ºC for one minute 15 seconds, 72ºC extension for one minute 30 seconds, and post extension at 72ºC for eight minutes and PCR final conditions 4^{o}C. The composition nucleotide bases SSR primers can be seen in Table 1.

Data analysis

Base pair (bp) sizes of the PCR products were determined using UVITEC Cambridge Fire Reader with a standard size of 100 bp (Invitrogen) DNA ladder, image data from electrophoresis to detect the appearance of DNA band. Data analysis was performed based on the scoring results of agarose gel. Calculation of the number of effective alleles per locus (Na), observed heterozygosity (Ho) and expected heterozygosity (He) was used by Gen Alex Ver. 6,501 (Peakall and Smouse, 2012). The SSR primer polymorphic information content (PIC) values were calculated by using Power Marker 3.25 software (Liu, 1998). Botstein *et al*. (1980) classify the PIC value into three classes, namely: PIC > 0.5: very informative; 0.25 > PIC < 0.5: while PIC < 0.25. Principal Coordinates Analysis (PCoA) was used to calculate the values of molecular diversity, and Neighbor-Joining Tree (NJTree) was used to find out the picture of kinship among individual populations, using DARwin Software version 6 (Perreira and Jacquemoud-Collet, 2014).

RESULTS

Phenotypic observations in soybean mutant lines

The data on phenotypic observations were combined with molecular observations to assist genetic identification in selecting M_6 soybean mutant lines resistant to stem rot disease, and to obtain more accurate results. Phenotype analysis was performed by calculating M_6 genotype means, and parental (Anjasmoro) and check (KipasPutih) genotype means and the t-value was calculated (Table 2).

Table 1. Sequences of primers used in the study.

No.	Primers	Forward Sequence (5′ - 3′)	Reverse Sequence (5′ - 3′)
1	Satt009	CCAACTTGAAATTACTAGAGAAA	CTTACTAGCGTATTAACCCTT
2	Satt114	GGGTTATCCTCCCCAATA	ATATGGGATGATAAGGTGAAA
3	Satt147	CCATCCCTTCCTCCAAATAGAT	CTTCCACACCCTAGTTTAGTGACAA
4	Satt191	CGCGATCATGTCTCTG	GGGAGTTGGTGTTTTCTTGTG
5	Satt197	CACTGCTTTTTCCCCTCTCT	AAGATACCCCCAACATTATTTGTAA

Table 2. Phenotypic data of eight soybean mutant lines.

Variable	Lines	Optimum Land			Disease Inoculation Land		
		Genotype Mean	Control Mean	T-Value	Genotype Mean	Control Mean	T-value
Flowering age	M100-A25(5/3)	37.88	37.03	1.66	36.98	37.42	1.17
	M100-A6(31/1)	37.66	37.03	7.50**	38.18	37.42	2.02*
	M200-A11(32/3)	36.91	37.03	1.01	37.07	37.42	0.89
	M200-A12(6/5)	35.54	37.03	5.52**	36.77	37.42	1.57
	M200-A17(13/6)	36.46	37.03	3.09**	36.82	37.42	2.04*
	M200-A17(18/5)	36.30	37.03	3.04**	36.73	37.42	1.85
	M300-A6(33/8)	36.52	37.03	2.12*	36.36	37.42	2.53*
	M300-A8(35/7)	36.75	37.03	1.23	36.14	37.42	3.73**
Harvest age	M100-A25(5/3)	96.36	100.95	6.67**	95.84	100.21	7.46**
	M100-A6(31/1)	95.80	100.95	9.96**	95.32	100.21	8.86**
	M200-A11(32/3)	95.02	100.95	9.59**	95.11	100.21	9.21**
	M200-A12(6/5)	95.32	100.95	10.94**	95.64	100.21	8.37**
	M200-A17(13/6)	95.81	100.95	9.84**	95.15	100.21	9.61**
	M200-A17(18/5)	95.78	100.95	9.34**	95.57	100.21	8.32**
	M300-A6(33/8)	95.59	100.95	9.95**	95.67	100.21	7.89**
	M300-A8(35/7)	96.07	100.95	9.11**	95.27	100.21	9.20**
Plant height	M100-A25(5/3)	56.69	55.27	0.49	56.15	55.40	0.34
	M100-A6(31/1)	58.78	55.27	1.32	70.76	55.40	7.45**
	M200-A11(32/3)	51.80	55.27	1.35	58.08	55.40	0.33
	M200-A12(6/5)	68.97	55.27	4.85**	59.49	55.40	1.79
	M200-A17(13/6)	55.62	55.27	0.13	59.55	55.40	1.69
	M200-A17(18/5)	58.5	55.27	1.16	60.97	55.40	2.35*
	M300-A6(33/8)	65.34	55.27	3.37**	66.27	55.40	5.02
	M300-A8(35/7)	57.42	55.27	0.81	60.05	55.40	2.29*
Productive branches (#)	M100-A25(5/3)	3.80	2.98	2.70**	3.38	2.82	1.94
	M100-A6(31/1)	3.46	2.98	1.54	3.70	2.82	3.09*
	M200-A11(32/3)	3.71	2.98	1.93	3.04	2.82	0.87
	M200-A12(6/5)	3.80	2.98	2.68**	3.66	2.82	3.01**
	M200-A17(13/6)	3.87	2.98	2.85**	2.93	2.82	0.39
	M200-A17(18/5)	3.15	2.98	0.56	3.43	2.82	2.12*
	M300-A6(33/8)	3.68	2.98	2.18*	3.20	2.82	1.38
	M300-A8(35/7)	3.48	2.98	1.60	3.96	2.82	3.95**
Pods plant^{-1}	M100-A25(5/3)	67.59	53.30	2.03*	60.14	55.76	0.71
	M100-A6(31/1)	55.84	53.30	0.40	60.43	55.76	0.83
	M200-A11(32/3)	68.91	53.30	2.40*	59.45	55.76	0.63
	M200-A12(6/5)	67.43	53.30	2.03*	52.96	55.76	0.48
	M200-A17(13/6)	65.17	53.30	1.79	52.85	55.76	0.47
	M200-A17(18/5)	70.61	53.30	2.58*	58.50	55.76	0.46
	M300-A6(33/8)	64.71	53.30	1.61	52.80	55.76	0.54
	M300-A8(35/7)	63.55	53.30	1.60	58.36	55.76	0.47
Total number of seed	M100-A25(5/3)	118.18	90.53	2.24*	112.88	99.32	1.18
	M100-A6(31/1)	101.89	90.53	0.97	119.32	99.32	1.94
	M200-A11(32/3)	115.75	90.53	2.08*	110.29	99.32	1.03
	M200-A12(6/5)	116.63	90.53	2.03*	98.32	99.32	0.09
	M200-A17(13/6)	125.72	90.53	2.8**	99.17	99.32	0.01
	M200-A17(18/5)	127.28	90.53	2.93**	107.2	99.32	0.73
	M300-A6(33/8)	108.13	90.53	1.42	92.83	99.32	0.65
	M300-A8(35/7)	115.32	90.53	2.11*	107.09	99.32	0.79
Seed weight plant^{-1}	M100-A25(5/3)	18.45	14.52	1.95	17.27	15.45	1.00
	M100-A6(31/1)	17.09	14.52	1.33	18.62	15.45	1.90
	M200-A11(32/3)	18.38	14.52	1.93	17.07	15.45	0.95
	M200-A12(6/5)	16.91	14.52	1.21	15.02	15.45	0.27
	M200-A17(13/6)	20.02	14.52	2.63*	16.01	15.45	0.30
	M200-A17(18/5)	19.21	14.52	2.30*	16.67	15.45	0.71
	M300-A6(33/8)	16.65	14.52	1.06	14.34	15.45	0.69
	M300-A8(35/7)	18.37	14.52	1.97	15.82	15.45	0.79
100-seed weight	M100-A25(5/3)	15.62	15.71	0.16	15.43	15.58	0.41
	M100-A6(31/1)	16.97	15.71	2.17*	15.78	15.58	0.65
	M200-A11(32/3)	15.80	15.71	0.17	15.5	15.58	0.35
	M200-A12(6/5)	14.79	15.71	1.64	15.32	15.58	0.63
	M200-A17(13/6)	15.90	15.71	0.32	16.36	15.58	2.07*
	M200-A17(18/5)	15.17	15.71	0.91	17.48	15.58	0.86
	M300-A6(33/8)	15.38	15.71	0.6	15.38	15.58	0.71
	M300-A8(35/7)	16.02	15.71	0.57	14.72	15.58	0.45

Phenotypic data analysis showed that on average the mutant lines revealed greater production than the parental cultivar. However, the average 100-seed weight of soybean M_6 lines M200-A17(13/6), M200-A17(18/5), and M200-A12(6/5) was lower in non-inoculated soil as compared to the same in fungus (*A. rolfsii*) inoculated soil. This might be due to the resistance of the above mutant lines to *A. rolfsii* and which helps out the plant to avoid stem rot disease which causes the yield losses. However, the 100-seed weight and production will decrease in soybean genotypes with no resistance to stem rot. Overall, the M_6 mutant lines had a better performance than parental cultivar.

Amplification of DNA soybean mutant line with SSR markers

DNA amplification of eight soybean M_6 mutant lines, parental genotype Anjasmoro and check cultivar KipasPutih for determining the resistance to stem rot (*A. rolfsii*) was carried out using five SSR primers. Three out of five primers used in this study were able to amplify the 10 tested plant DNA. Two banding patterns were obtained around 124 bp to 415 bp indicating the heterozygous bands. Visualization of the results at 2% agarose gel electrophoresis with five SSR markers is presented in Figure 1.

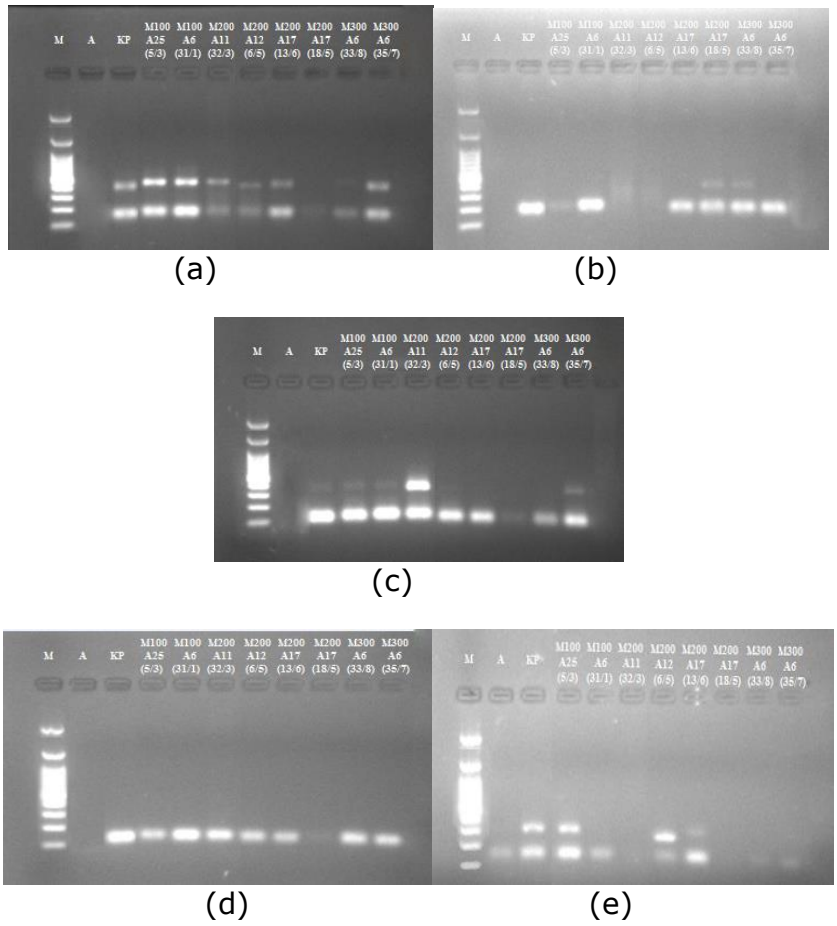

Figure 1. The amplification pattern of SSR markers of mutant lines studied for resistance to *A. rolfsii* with primers (a) Satt009, (b) Satt114, (c) Satt147, (d) Satt191, and (e) Satt197.

Table 3. Profile of five SSR markers on 10 soybean individuals studied for resistance to *A. rolfsii*.

No.	Primers	Linkage Group (LG)	cM Position in LG	Number of alleles detected	Allele size (bp)	PIC
1	Satt009	N	28.52	5	170 - 400	0.29
2	Satt114	D1a	108.89	5	215 - 415	0.29
3	Satt147	F	63.69	10	124 - 400	0.52
4	Satt191	G	96.57	5	126 - 159	0.29
5	Satt197	B1	46.39	6	171 - 345	0.34

Soybean cultivar KipasPutih is resistant to *A. rolfsii* and has broad adaptation than the other soybean cultivars. Based on present results of the soybean mutant DNA visualization using five specific primers to detect genes that control the resistance to *A. rolfsii*, it appeared that some soybean M_6 mutant lines have same banding pattern as found in the resistant cultivar KipasPutih.

The SSR primer Satt009 was used to genotype the seven M_6 lines i.e., M100-A25(5/3), M100-A16(31/1), M200-A11(32/3), M200-A12(6/5), M200-A17(13/6), M300-A6(33/8), and M300-A8(35/7) and data were recorded. The primer Sat114 could discriminate six lines namely M100-A25 (5/3), M100-A6 (31/1), M200-A17 (13/6), M200-A17(18/5), M300-A6(33/8), and M300-A8 (35/7). In primer Satt147 the recorded lines were seven i.e., M100-A25(5/3), M100-A16(31/1), M200-A11(32/3), M200-A12(6/5), M200-A17(13/6), M300-A6(33/8), and M300-A8(35/7). The primer Satt 191 was used to identify seven lines namely M100-A25(5/3), M100-A16(31/1), M200-A11(32/3), M200-A12(6/5), M200-A17(13/6), M300-A6(33/8), and M300-A8(35/7). SSR primer Satt197 was used to profile the four soybean lines i.e., M100-A25 (5/3), M100-A6(31/1), M200-A12 (6/5), and M200-A17 (13/6).

SSR band profile and polymorphic informative content

PIC values for the SSR primers ranged from 0.29 to 0.52. The most informative marker to identify the stem rot disease in soybean due to the highest PIC value (0.52) was SSR primer Satt147 and its marker profile is shown in Table 3.

Heterozygosity and statistical test for codominant SSR markers

Genetic identification of the individual soybean M_6 mutant lines resistant to *A. rolfsii* showed that an average value of observed heterozygosity (Ho = 0.244) was lower than the expected average heterozygosity (He = 0.317). The statistical test for codominant markers is shown in Table 3.

Principal coordinates analysis (PCoA)

The PCoA analysis showed that there are four different axis. The amount value of the axis-1 and axis-2 (75.98%) revealed the molecular diversity of five SSR primers used in 10 soybean DNA. The PCoA was used to map the individuals in the population. The PCoA results among the parental cultivar Anjasmoro, check genotype KipasPutih and M_6 mutant lines can be seen in Figure 2.

Phylogenetic analysis of soybean mutant lines for resistance to *A. rolfsii*

Based on the present results from dissimilarity analysis, there were four main groups. Group I consisted of four individuals namely, KipasPutih, M100-A25(5/3), M100-A6(31/1), and M200-A17(13/6). Group II contained one individual namely M200-A12(6/5). Group

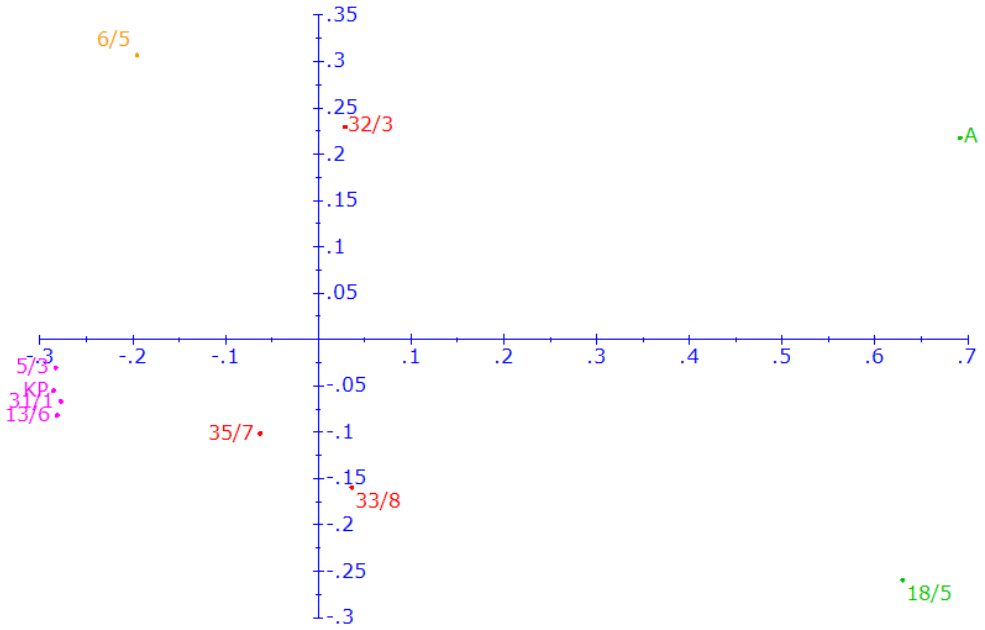

Figure 2. Principal coordinate analysis (PCoA) of parental cultivar Anjasmoro, check genotype KipasPutih and mutant lines for resistance to *A. rolfsii*.

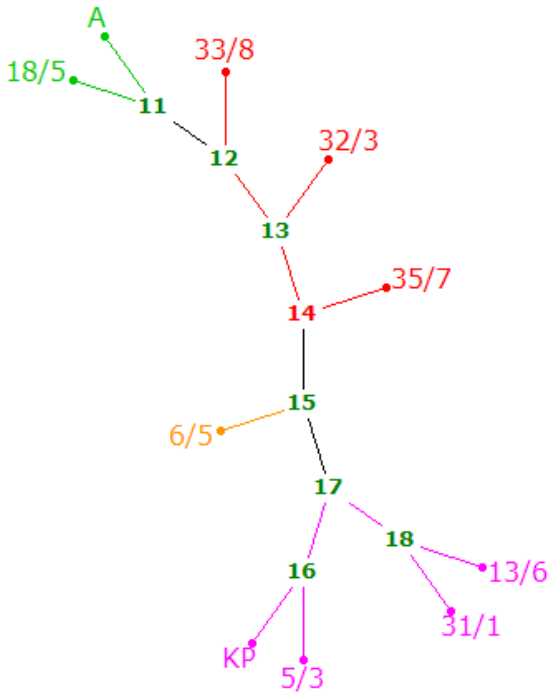

Figure 3. Radial Neighbor-Joining Tree (NJtree) Profile of 10 soybean DNA analyzed based on matrix dissimilarity simple matching using SSR markers.

III comprised of three individuals i.e., M200-A11 (32/3), M200-A6(33/8), and M300-A8 (35/7). Group IV consisted of two individuals namely Anjasmoro, and M200-A17(18/5). This grouping was very important information for plant breeding process, where the assembly of the new genotypes could be more effective if selected from different groups. Phylogenetic analysis of soybean M_6 mutant lines along with parental and check cultivars can be seen in Figure 3.

DISCUSSION

Based on phenotypic observations, the varied values were recorded among M_6 soybean mutant lines for growth and development traits (Table 2). Some of phenotypic characters were basically controlled by polygenes. Both qualitative and quantitative phenotypic characters resulted from growth and development processes that are directly related to physiological and morphological characters. The phenotypic characters like yield and its associated traits showed differences in the final productivity among the M6 mutant lines and parental and check genotypes. Based on observations, several changes were recorded due to the induction of mutation with gamma rays irradiation, and the mutant lines flower more earlier than their parental genotype. These changes might be due alteration in the genetic make-up of the individual mutant lines due to irradiation. Consistent with Chahal and Gosal (2006), mutation results in changes in phenotype of mutant crop plants in the form of phenotypic traits.

DNA amplification is a molecular process that enhancing the copying of a chromosomal region and often causes elevated expression of the amplified genes (Mukherjee and Storici, 2012). DNA amplification resulted only several individual mutant lines was amplified by five SSR primers which exhibited that primer is specific towards the resistance to *A. rolfsii*. Kusumadewi (2011) findings revealed that there were bands generally found in all the mutants, however, some specific markers were recorded only in certain mutant lines and species.

The studied primer Satt147 amplified the highest number of markers, while primers Satt009, Satt114, and Satt191 amplified the lowest number of DNA bands. Unamplified DNA were incompatible with DNA template so that the primers did not bind to the target sequence. According to Harahap (2014), nucleotide bases will only bind to the target sequence. Experimental evidence has shown that a difference in one base pair is enough to cause a mismatch of the primer template which prevents amplification. Besides, the SSRs can be amplified by tandard polymerase chain reaction (PCR), using specific primer sequences from the flanking regions (Saavedra *et al*., 2013; Stolle *et al*., 2013).

Results obtained from the analysis of five primers on the *A. rolfsii* resistance produced a number of alleles per primer with varying range and length of alleles. Based on a single primer, the number of alleles ranged from 5 to 10. The highest number of alleles was detected by primer Satt147 with 10 alleles, while the least number of alleles were observed in primers Satt009, Satt114, and Satt191, with only five alleles. The longest allele measuring between 215 to 415 base pairs (bp) obtained in the primer Satt114, while the shortest allele (126 to 159 bp) was obtained in the primer Satt191. This difference in the number of alleles indicates genetic variation between soybean mutant lines that occur due to mutation. The SSRs are codominant molecular markers that distinguish homozygous and heterozygous individuals and also take a large number of alleles. In fact, the past studies revealed that the use of single SSR marker may not provide authentic information in maize, and hence, the three different SSR markers were used for reliable and accurate differentiation (Shehata *et al*., 2009). The SSR markers can be used to identify the different alleles among the mutant lines from the same origin.

Table 4. Parameters of genetic diversity with five SSR markers used in three soybean populations for resistance to *A. rolfsii*.

Populations	Individual		N	Na	Ne	Ho	He
Anjasmoro	1	Mean	0.000	0.000	0.000	0.000	0.000
		SE	0.000	0.000	0.000	0.000	0.000
KipasPutih	1	Mean	3.000	1.400	1.400	0.400	0.200
		SE	0.000	0.245	0.245	0.245	0.122
Mutant lines	8	Mean	18.600	5.400	4.522	0.331	0.750
		SE	1.749	1.208	0.931	0.150	0.036
Total	10	Mean	7.200	2.267	1.974	0.244	0.317
		SE	2.245	0.720	0.586	0.100	0.093

Note: N = Number of samples; Na = Average number of alleles; Ne = Average number of effective alleles; Ho = average heterozygosity of observations; He = Average expectations heterozygosity.

Primers used were included in the informative class as indicated by an average PIC value ≥ 0.5, whereas primers that have large PIC values were considered as the best primers which can be used as molecular markers. According to Elston (2005), the value of polymorphic information is directly proportional to the number of alleles in each locus. The higher the number of alleles, the higher the polymorphic information value. Markers with very large numbers of alleles tend to have higher PIC values and become more informative.

Statistical analysis of codominant data on soybean mutant lines using five SSR primers showed that the average number of effective alleles (Ne = 1.974) was lower than the average number of observed alleles (Na = 2.267) (Table 4). This showed that there were more homozygous individuals in soybean mutant lines. Solin *et al*. (2013) stated that Ne shows the number of effective alleles which is opposite to the homozygosity values. The higher the value of Ne, the more the number of heterozygous individuals. This study was also supported by Rell *et al*. (2013), and mentioned that heterozygosity is one of the parameters used to measure the level of genetic diversity in a population based upon the allele frequency at each locus.

The number of identified populations of a given species often does not correspond to the number of actual genetic groups. An analysis of PCoA diagrams revealed that markers split the studied populations into two groups (micro-satellite loci) or three groups (ISJ loci) which, however, differed in the populations that created them (Bilska and Szczecinska, 2016). These differences in PCoA diagrams for neutral SSR markers and putatively non-neutral markers were reported by Loywyck *et al*. (2008) (SSR markers in QTL regions) and Singh *et al*. (2013) (SNP markers developed from conserved single-copy genes).

In this study, the disease severity of *A. rolfsii* to KipasPutih was low. However, the mutant lines showed the greater adaptation to *A. rolfsii* with different level of intensity caused by genetic change. These results were in line with the findings of Hanafiah *et al*. (2020), who reported that the intensity of stem rot disease attack on each soybean genotype was i.e., cultivar KipasPutih (19.05%), Anjasmoro (45.24%), M100A25(5/3) (8.93%), M200A11(32/3) (7.14%), M100A6(31/1) (38.10%), M300A8(35/7) (10.36%), M200A17(13/6) (19.64%), M200A17(18/5) (11.07%), M200A12(6/5) (32.14%), and M300A6 (33/8) (10.00%). The differences in the intensity of stem rot attack might be due changes in the genetic make-up of the individual soybean mutant lines which have been irradiated with different doses.

In this study, soybean mutant lines amplified by five specific primers only consisted of three lines, namely M100-A25(5/3), M100-A6(31/1), and M200-

A17(13/6). However, only mutant line M200-A17(13/6) revealed the same DNA banding pattern as already found in the resistant and check soybean cultivar KipasPutih. This showed that the lines have specific DNA fragment patterns that were able to be amplified by the three primers. According to Subositi and Widiyastuti (2013), each DNA sample has a specific DNA fragment pattern, and the DNA fragments that appear in certain DNA samples can be used as markers for characterization and identification of different crop plant accessions.

CONCLUSIONS

Molecular analysis identified the soybean mutant line [M200-A17 (13/6)] with resistance to *A. rolfsii* by using specific primers. The present research could be helpful and identified a mutant line could be used as source material for developing high yielding soybean cultivars with resistance to stem rot disease (*A. rolfsii*) to enhance the national production.

ACKNOWLEDGEMENTS

The authors wish to thank Rector of the Universitas Sumatera Utara and ministry of Research. Technology and Higher Education. Republic of Indonesia that has supported and funded this research project.

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