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Genetic Variation in Selected Individuals Based on Number of Capsules in M5 Sesame Mutant Lines Detected by RAPD

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ABSTRACT
Sesame (*Sesamum indicum* L.) is major oilseed crops with advantages in health and food industry. Due to self pollinated crop, breeding program in sesame utilized gamma rays irradiation to increase genetic variation. The research material consisted of 164 genotypes from 22 selected individual mutant line based on the number of capsules in M5 generation. This study is to detect genetic variation in selected individuals based on number of capsules in M5 generation of sesame mutant line using RAPD markers. The analysis consists of percentage of polymorphic loci, analysis of molecular variance and visualized in cluster and co-ordinate analysis. Fifteen primers RAPD were able to amplified 237 loci. Each genotype in populations had the similarity coefficient of 0.29 – 0.85. Variance within selected individual line (66%) was higher than variance among selected mutant lines (34%). Variance in each selected individual line contributed to its high value. Line 34 showed the lowest polymorphism (23.21%) and line 19 depicted the highest polymorphism (61.60%).

Keywords: Molecular marker, mutation, polymorphism.

INTRODUCTION

Sesame is cultivated for its oil content. The unsaturated fatty acids composed about 80% of sesame oil and its beneficial for health. The lignin contained in sesame oil caused the resistance to oxidation and gave long shelf life. Sesame and other plants belong to the genus Sesamum and the Pedaliaceae family demonstrated very wide range of adaptability to environmental conditions. Sesame thrives in soil with low fertility and limited water availability (Bedigian, 2010; Weiss, 1971). In Indonesia, sesame is mostly used in food industry as the whole seed in bakery or as cooking oil.

Genetic variation in self-pollinated crops such as sesame is low. Mutation is one of methods to generate genetic variation (Acquaah, 2012). Mutations occured randomly throughout the genome and within the locus or genes, thereby not only producing desired mutation but allowing the emergence of other mutations that can increase the diversity of a germplasm. Induced mutations with gamma ray irradiation are often performed due to their wide availability and flexibility of use (Foster & Shu, 2012).

Homogeneous seeds of black and white sesame seeds were treated with 100 – 800 Gy 60Co gamma rays. M2 generation populations are screened with 6 gl^{-1} NaCl solution for salinity tolerance and the results were planted to produce M3 generation (Pramujari, 2015, personal comm.). Further selection which based on plant height and number of capsules per plant in M4 resulted in significant decreased of genetic variability of seed yield and yield components in M5 generations of sesame mutant lines (Aristya, 2017).

Genetic variance detection with molecular markers offered several advantages over morphology, as they were not confounded by environmental and able to detect polymorphism in DNA level (Zainudin *et al*., 2014). Random Amplified Polymorphic DNA (RAPD) is one of methods in molecular markers based on amplification with Polymerase Chain

Reaction (PCR). Several study in genetic variation using RAPD in sesame are conducted by Bhat *et al*. (1999) and Ercan *et al*. (2004), while Taryono *et al*. (2011) using RAPD to detect mutational changes in sorghum. This study is to detect genetic variation in selected individuals based on number of capsules in M5 generation of sesame mutant lines.

MATERIALS AND METHODS

The entire research work was carried out at Genetics and Plant Breeding Laboratory, Faculty of Agriculture, Universitas Gadjah Mada during the year 2016–2017.

Plant Material

Twenty two selected individuals based on number of capsules from M5 generation were used in this research. Each of selected individuals were grown in separated rows. The samples were randomly collected from each line and the total were 164 individual samples (Table 1).

DNA Isolation

DNA was extracted from 0.1 mg well-developed leaves using CTAB method (Doyle & Doyle, 1990). The total DNA were extracted with CTAB buffer and then diluted it to obtain final concentration of 2.5 ng μL^{-1}

RAPD Analysis

This research used 15 primers for RAPD analysis based on polymorphic bands. PCR touchdown was carried out to optimize the amplification. The initial heating was performed at 95ºC for 5 minutes and then followed by 15 cycles of denaturation at 95ºC for 15 sec; annealing at 41ºC for 30 sec; and elongation at 72ºC for 90 sec then repeated for the annealing temperatures at 39ºC and 37ºC and followed by final elongation at 72ºC for 7 minutes. Amplification was carried out in thermal cycler BioRad T100TM. The amplification results were analyzed by electrophoresis on 1.5% agarose gels 1st BASE stained with Floro Safe 1st BASE and photographed under ultraviolet light. Molecular weights were estimated using a 100 bp DNA ladder.

Data Analysis

Each band visualized in gel electrophoresis scored for 1 for the presence and 0 for the absent of

Table 1. Population of samples for RAPD analysis

Treatment	Selected Individual mutant	Sample number	Population Size
Control	39	12	12
100 Gy	17	11	45
	18	6	
	19	13	
	20	9	
	21	6	
300 Gy	23	7	16
	24	5	
	25	4	
400 Gy	26	9	19
	27	10	
500 Gy	28	10	30
	29	11	
	30	4	
	31	5	
600 Gy	32	5	5
700 Gy	33	7	20
	34	4	
	35	9	
800 Gy	36	7	17
	37	7	
	38	3	
			Σ = 164

Table 2. Amplification Result with 15 Primers RAPD

Primer	Sequence (5'- 3')	Number of loci Amplified	Band Size (bp)	% Polymorphism
OPA 7	GAAACGGGTG	19	250 – 2500	100
OPA 10	GTGATCGCAG	16	200 – 1700	100
OPB 7	GGTGACGCAG	17	350 – 2500	100
OPB 8	GTCCACACGG	11	300 – 1700	100
OPB 11	GTAGACCCGT	9	300 – 1500	100
OPB 12	CCTTGACGCA	14	200 – 2000	100
OPB 18	CCACAGCAGT	15	200 – 1700	100
OPC 4	CCGCATCTAC	17	250 – 2000	100
OPC 5	GATGACCGCC	17	250 – 2000	100
OPC 8	TGGACCGGTG	17	300 – 2000	100
OPC 11	AAAGCTGCGG	17	200 – 3000	100
OPC 12	TGTCATCCCC	19	250 – 2000	100
OPC 16	CACACTCCAG	16	270 – 2500	100
OPD 12	CACCGTATCC	15	300 – 2500	100
OPD 20	ACCCGGTCAC	18	250 – 2000	100
		Σ = 237		

Table 3. Percentage of Polymorphic Loci in Selected Individual with 15 Primers RAPD

Selected Individual mutant (line)	Percentage of Polymorphic Loci (%)
39 (control)	50.21
17	50.21
18	46.41
19	61.60
20	54.85
21	45.99
23	52.32
24	46.41
25	33.76
26	45.57
27	45.99
28	47.68
29	49.37
30	36.71
31	49.37
32	40.51
33	50.21
34	23.21
35	52.74
36	54.43
37	37.97
38	25.32

bands. Analysis of percentage of polymorphic loci, Analysis of Molecular Variance (AMOVA) and Principal Coordinate Analysis (PCoA) was carried out with GenAlEx 6.1. Coefficient genetic similarity and cluster analysis which based on Unweighted Pair Group Method with Arithmatic (UPGMA) was carried out with NTSYSpc v 2.02.

RESULTS AND DISCUSSION

Amplification results based on 15 primer RAPD showed 100% polymorphism and were able to amplify 237 loci (Table 2). The average number of loci being amplified was 15.8 for each primer. In primer OPB 8 (Abdellatef *et al*., 2008) and OPC 4 (Salazar *et al*., 2006) also produced 100% polymorphism and were able to amplify 6 and 7 loci. The number of loci that was formed depent on how the primer recognizes the homologous DNA template. The more homologous DNA sites with primers, the more number of loci are amplified (Poerba & Yuzammi, 2008). Figure 3 showed polymorphic bands as shown by the arrows among samples from selected individual 28 using primer OPA 10.

The percentage of polymorphic loci is used to determine the variability in the group (Laurentin, 2009). In this study, the percentage of polymorphic loci at the highest selected numbers was in line 19 with 61.60%, while the lowest was in line 34 with 23.21% (Table 3). Genetic variation in line 19 was considered as the biggest contributor for the variation

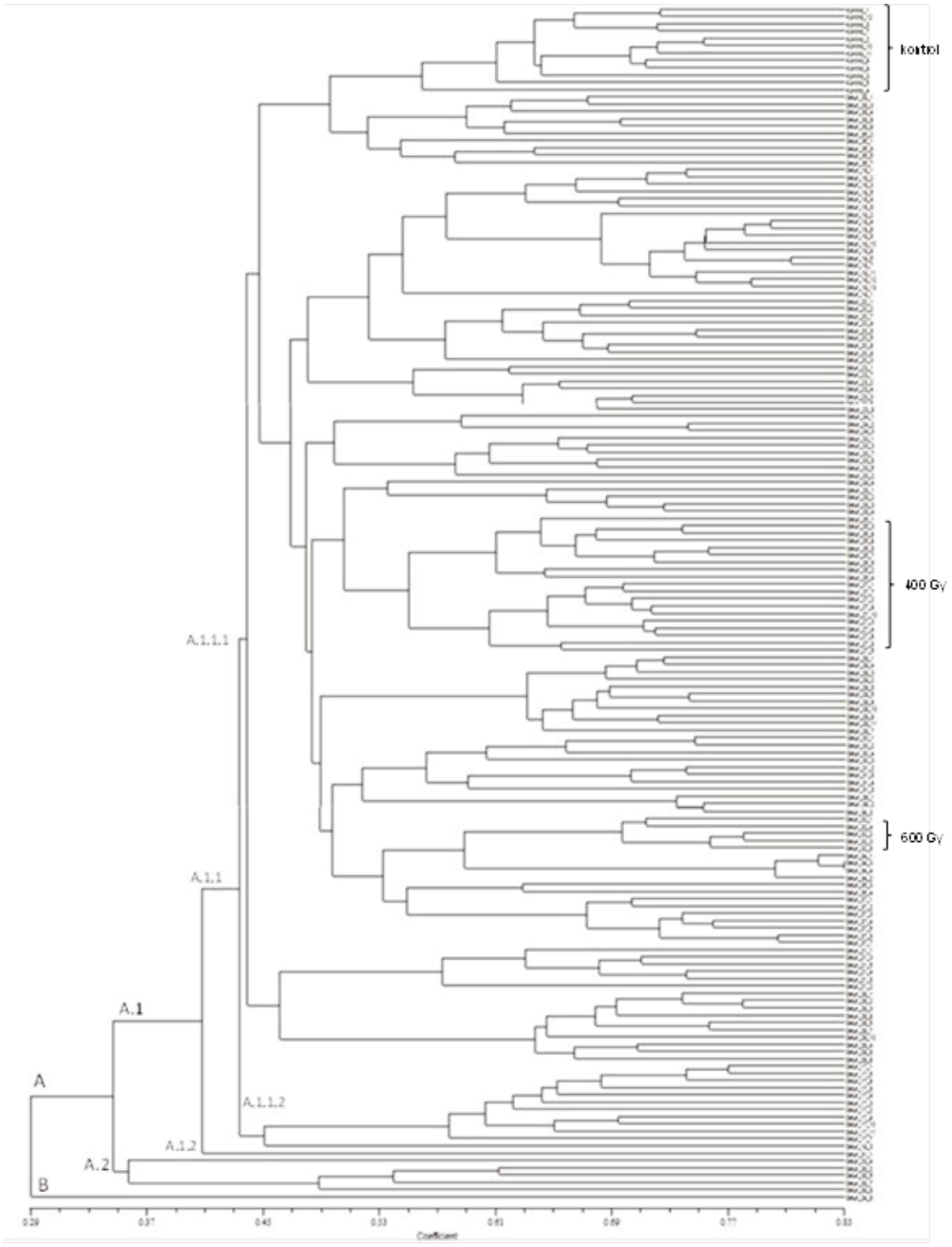

Figure 1. Dendrogram of 164 selected individual lines

Table 4. AMOVA for Selected Individual

Source of Variance	df	SS	MS	Est. Var.	(%)
Between Selected Individual	21	2365.338	112.635	12.105	34
Within Selected individual	142	3268.009	23.014	23.014	66
Total	163	5633.347		35.119	100

in 100 Gy dose treatment. Genetic variation within selected individual lines was higher than genetic variation among selected mutant lines (Table 4).

The similarity coefficient ranged between 0.29 – 0.85 (Figure 1). Two genotypes from line 34 showed the highest similarity coefficient with 0.85. The cluster analysis divided 164 genotypes into two major groups A and B at similarity level 0.29. Group B consisted of one genotypes from line 24 and group A consists of 163 genotypes from all selected individuals. Group A was divided into three subgroups at similarity level 0.35; 0.41; and 0.43. Among subgroup at similarity level 0.43, there were four selected individual lines clustered with different similarity level (line 39, 32, 27, and 26). This cluster analysis was also supported with PCoA (Figure 2) that several selected individual lines grouped into the same quadrant. Line 32 grouped into quadrant III and line 39 grouped into quadrant I.

Mutation is the ultimate source of variation and may be caused by alteration in DNA replication or damage by radiation (de Vicente *et al*., 2004). Variation in DNA can be used as a source of polymorphism. In this study, 15 primers of RAPD were able to detect polymorphism in DNA level. Based on percentage of polymorphic loci, line 34 showed the lowest polymorphism and the highest polymorphism detected in line 19 with 61.60%. This result was also supported by the estimated variance in Analysis of Molecular Variance (AMOVA) that genetic variation within selected individual line higher (66%) than among selected mutant lines (34%).

Gamma rays are a class of ionizing radiation which produce free radicals in cells that are capable of modify cells components and consequently affect different morphological, anatomical, biochemical and physiological characters of plants depending on the dose of mutation (Gafaar *et al*., 2016). Diverse genetic

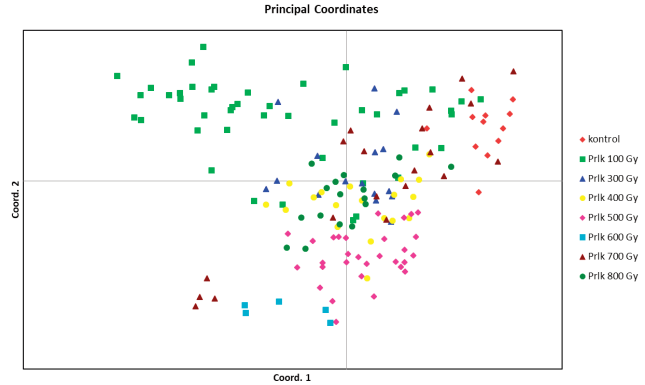

Figure 2. PCoA of 164 individual sample plants of 22 selected individual mutant lines

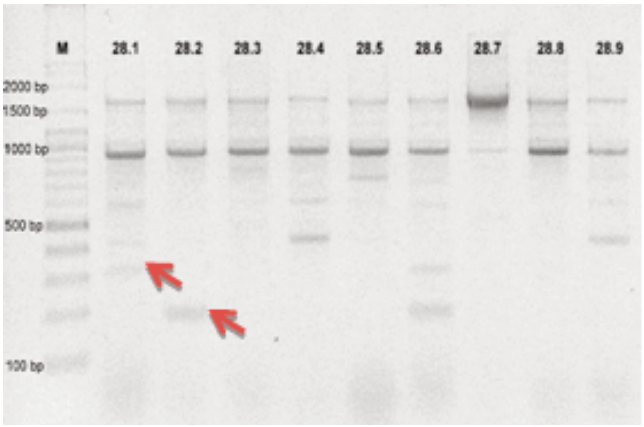

Figure 3. Amplification result from primer OPA 10 in selected individual 28

variation as a result from different dose of gamma irradiation was recorded in wheat (Indriatama *et al*., 2016) and lentil (Laskar & Khan, 2017).

Sesame is a self pollinated plants and often have lower genetic variability than outcrossing plants, and that variability tends to be found between populations (Charlesworth, 2003). In this research, AMOVA is proposed to identify molecular variation within and between population (selected individual lines). In this study, genetic variation within selected individual lines higher than among selected individual lines. In self pollinated plants, subsequent generations tend to have increased homozygous level. If the pair of genes is more than one, the decreased in heterozygous level will not be as fast as if there only one pair of genes (Mangoendidjojo, 2003). In this study, selection is based on the number of capsule from M4 generation. The number of capsule is quantitative traits that are controlled by many genes (polygenic) and each of it has little effect on expression of a plant character (Kasno & Trustinah, 1998).

CONCLUSIONS

The distribution value of genetic variation within selected individual line (66%) was higher than among selected individual lines (34%). This variation resulted polymorphism which can be detected with RAPD markers. Line 34 showed the lowest polymorphism (23.21%) whereas line 19 depicted the highest polymorphism (61.60%). Each genotype in population had the similarity coefficient of 0.29 – 0.85. This high genetic diversity within selected individual line due to different effect of dose gamma irradiation and selection based on the number of capsule as of needs further selection in subsequent generations.

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